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Molecular Interactions with Mercury in the Kidney

RUDOLFS K. ZALUPS[1]

Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, Georgia

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Abstract——**Mercury is unique among the heavy metals in that it can exist in several physical and chemical forms, including elemental mercury, which is a liquid at room temperature. All forms of mercury have toxic effects in a number of organs, especially in the kidneys. Within the kidney, the pars recta of the proximal tubule is the most vulnerable segment of the nephron to the toxic effects of mercury. The biological and toxicological activity of mercurous and mercuric ions in the kidney can be defined largely by the molecular interactions that occur at critical nucleophilic sites in and around target cells. Because of the high bonding affinity between mercury and sulfur, there is particular interest in the interactions that occur between mercuric ions and the thiol group(s) of proteins, peptides and amino acids. Molecular interactions with sulfhydryl groups in molecules of albumin, metallothionein, glutathione, and cysteine have been implicated in mechanisms involved in the proximal tubular uptake, accumulation, transport, and toxicity of mercuric ions. In addition, the susceptibility of target cells in the kidneys to the injurious effects of mercury is modified by a number of intracellular and extracellular factors relating to several thiol-containing molecules. These very factors are the theoretical basis for most of the currently employed therapeutic strategies. This review provides an update on the current body of knowledge regarding the molecular interactions that occur between mercury and various thiol-containing molecules with respect to the mechanisms involved in the renal cellular uptake, accumulation, elimination, and toxicity of mercury.**

I. Introduction

Among metals, mercury is unique in that it is found in the environment in several physical and chemical forms. At room temperature, elemental (or metallic) mercury exists as a liquid. As a result of its high vapor pressure, this form of mercury is released into the environment as mercury vapor. Mercury also exists as a cation with an oxidation state of 1+ (mercurous) or 2+ (mercuric). In occupational and environmental settings, the most common cationic form of mercury encountered is the mercuric form, which may have a valence of 1+ or 2+, depending on whether the mercuric ion is covalently bonded to a carbon atom of an organic side group, such as an alkyl group. With respect to organic forms of mercury, methylmercury is the most frequently encountered organic mercuric compound in the environment. It forms mainly as the result of methylation of inorganic (mercuric) forms of mercury by microorganisms in soil and water.

Due to industrialization and changes in the environment during the twentieth century, humans and animals are exposed to numerous chemical forms of mercury, including elemental mercury vapor (Hg^0), inorganic mercurous (Hg^+) and mercuric (Hg^{2+}) compounds, and organic mercuric ($R\text{-}Hg^+$ or R-Hg-R; where R represents any organic ligand) compounds (Fitzgerald and Clarkson, 1991). Inasmuch as mercury is ubiquitous in the environment, it is nearly impossible for most humans to avoid exposure to some form or forms of mercury on a regular basis.

All forms of mercury cause toxic effects in a number of tissues and organs, depending on the chemical form of mercury, the level of exposure, the duration of exposure, and the route of exposure. The kidneys are the primary target organ where inorganic mercury is taken up, accumulated, and expresses toxicity. Organic mercuric compounds are also nephrotoxic but to a lesser degree than inorganic mercurous or mercuric compounds. Systemic distributions of organic mercury are more diffuse than inorganic forms, and they affect other target organs, including hematopoietic and neural tissues (Clarkson, 1972; World Health Organization, 1991; Agency for Toxic Substance and Disease Registry, 1999). Differences in the mechanisms involved in the transport and metabolism of inorganic and organic forms of mercury (in the various compartments of the body) are likely responsible for the disparity in their distribution in tissues and organs, pattern of biological effect, and toxicity (Zalups and Lash, 1994).

When considering the biological activity of mercuric ions in humans or other mammals, one must take into account the bonding characteristics of these ions. Although mercuric ions will bind to numerous nucleophilic groups on molecules, they have a greater predilection to bond to reduced sulfur atoms, especially those on endogenous thiol-containing molecules, such as glutathione, cysteine, homocysteine, *N*-acetylcysteine, metallothionein, and albumin. The affinity constant for mercury bonding to thiolate anions is on the order of 10^{15} to 10^{20}. In contrast, the affinity constants for mercury bonding to oxygen- or nitrogen-containing ligands (e.g., carbonyl or amino groups) are about 10 orders of magnitude lower. Hence, it is reasonable, in most cases, to consider the biological effects of inorganic or organic mercury in

[1] Address for correspondence: Dr. Rudolfs K. Zalups, Division of Basic Medical Sciences, Mercer University School of Medicine, 1550 College Street, Macon, GA 31207. E-mail: ZALUPS.RK@GAIN.MERCER.EDU

terms of their interactions with sulfhydryl-containing residues.

In the presence of an excess of a low-molecular-weight thiol-containing molecule, mercuric ions have a high propensity toward linear II coordination with two of these molecules. For example, in a situation in which there are twice as many molecules of glutathione as inorganic mercuric ions in aqueous solution (at room temperature), there will be a strong tendency for each mercuric ion to form a linear II coordinate covalent complex with two molecules of glutathione by bonding to the sulfur atom on the cysteinyl residue of each of those two molecules (Fuhr and Rabenstein, 1973; Rabenstein, 1989). Organic mercurials, such as methylmercury, tend to form 1:1 complexes with thiol-containing molecules. Despite the thermodynamic stability of the (linear I or II) coordinate covalent bonds formed between mercuric ions and various thiol-containing molecules in aqueous solution, the bonding characteristics between mercuric ions and these thiol-containing molecules appear to be more labile within the living organism (Rabenstein, 1989). Complex factors such as thiol- and/or other nucleophilic competition and exchange are likely the most cogent explanation for the perceived labile nature of bonding that occurs between mercuric ions and certain thiol-containing molecules in particular tissue and cellular compartments. For example, most of the mercuric ions present in plasma (shortly after exposure to inorganic mercury) are bound to sulfhydryl-containing proteins, such as albumin (Friedman, 1957; Mussini, 1958; Cember et al., 1968; Lau and Sarkar, 1979). However, these mercuric ions do not remain bound to these proteins for very long. During the initial hours after exposure to inorganic mercury, there is a rapid decrease in the plasma burden of mercury concurrent with a rapid rate of uptake of inorganic mercury in the kidneys and liver. Because current evidence (to be discussed) indicates that mercuric *S*-conjugates of small endogenous thiols (e.g., glutathione and cysteine) are likely the primary transportable forms of mercury in the kidneys, it must be that mercuric ions are transferred from the plasma proteins to these low-molecular-weight thiols by some form of currently undefined complex ligand-exchange mechanism or mechanisms. Moreover, the effectiveness of thiol-containing pharmacological agents, such as penicillamine, *N*-acetylpenicillamine, *meso*-2,3-dimercaptosuccinic acid (DMSA),[2] 2,3-dimercapto-1-propanesulfonic acid (DMPS), dithioerythritol, and dithiothreitol, in reversal of or protection against toxic effects of mercury-containing compounds is fundamentally premised on, and best explained by, the ability of these agents to remove inorganic and organic mercuric ions from endogenous ligands via nucleophilic competition and exchange, thereby forming new thiol-mercury complexes.

Dose-response relationships for the toxicity of inorganic mercury are extremely steep in a variety of renal systems, including in vivo treatment of rats and rabbits (Zalups and Diamond, 1987b; Zalups et al., 1988; Zalups and Lash, 1990; Zalups, 1991c), renal cortical slices (Ruegg et al., 1987) and isolated segments of proximal tubules from rabbits (Barfuss et al., 1990; Zalups et al., 1993a), freshly isolated proximal tubular cells from rats (Lash and Zalups, 1992), and primary cultures of renal cortical cells from rats (Smith et al., 1986; Lash et al., 1999). In all of these various renal systems, a threshold effect is generally observed, in that no cellular necrosis (death) is observed up to a certain dose. Above that dose, however, cellular death progresses rapidly, and in some systems an all-or-none response is observed. This does not mean that subtoxic doses of mercury do not have biochemical or physiological effects. One possible explanation for the threshold effect and the subsequent steep dose-response curve is that endogenous ligands, such as glutathione, bind mercury and may act as a buffer to prevent functional changes from occurring. Above a certain dose or concentration of mercury, the buffer becomes depleted, and mercuric or mercurous ions can bind more readily to critical nucleophilic groups in the cell, thereby causing functional impairment. Intracellular sulfhydryl-containing proteins such as metallothionein or low-molecular-weight thiols, in particular glutathione, likely function in such a capacity.

To understand the nephropathy induced by mercury and to find therapeutic regimens to treat this nephropathy, it is essential to understand the mechanisms involved in the uptake, intracellular binding, and cellular elimination of mercury in the target cells, namely the epithelial cells lining the proximal tubule. In addition to seeking a better understanding of the chemical properties of mercury-containing compounds and the intracellular buffering capacity of both target and nontarget organs, other factors must be considered to define more precisely the biochemical and molecular mechanisms of action of mercury-containing compounds in the kidney. Particular attention must be paid to the potential role of "molecular mimicry" and the species of mercury involved in the renal (proximal) tubular uptake and transport of mercuric ions. Susceptibility to the injurious effects of mercury may be modified by a number of intracellular and extracellular factors. These very factors are the theoretical basis for most of the currently used therapeutic strategies. Physiological or pathological alterations in cellular function, particularly in the kidney and liver, may also play important roles in modifying susceptibility to mercury-induced renal injury. Consideration of these factors can provide clues that will aid in understanding the basic mechanisms of mercury-induced renal cellular injury.

[2] Abbreviations: DMSA, meso-2,3-dimercaptosuccinic acid; BUN, blood urea nitrogen; γ-GCS, γ-glutamylcysteine synthetase; γ-GT; γ-glutamyltransferase; DMPS, 2,3-dimercaptopropane-1-sulfonate; GFR, glomerular filtration rate; LDH, lactate dehydrogenase; NPX, nephrectomized.

II. Renal Disposition and Transport of Mercury

In humans and other mammals, the kidneys are the primary targets where mercuric ions accumulate after exposure to elemental or inorganic forms of mercury (Adam, 1951; Ashe et al., 1953; Friberg, 1956, 1959; Rothstein and Hayes, 1960; Berlin and Gibson, 1963; Clarkson and Magos, 1967; Swensson and Ulfvarson, 1968; Cherian and Clarkson, 1976; Zalups and Diamond, 1987a,b; Hahn et al., 1989, 1990; Zalups and Barfuss, 1990; Zalups, 1991a,b,c, 1993a). Renal uptake and accumulation of mercury in vivo are very rapid. As much as 50% of a low (0.5 μmol/kg) dose of inorganic mercury has been shown to be present in the kidneys of rats within a few hours after exposure (Zalups, 1993a). Significant amounts of mercury also accumulate in the kidneys after exposure to organic forms of mercury (Prickett et al., 1950; Friberg, 1959; Norseth and Clarkson, 1970a,b; Magos and Butler, 1976; Magos et al., 1981, 1985; McNeil et al., 1988; Zalups et al., 1992). However, the level of accumulation is much less than that which occurs after exposure to inorganic or elemental forms of mercury. For example, only about 10% of the administered dose of mercury has been shown to be present in the combined renal mass of rats 24 h after the administration of a non-nephrotoxic (5 mg/kg) dose of methylmercury (Zalups et al., 1992).

A. Intrarenal Distribution and Localization of Mercury

Within the kidneys, both inorganic and organic forms of mercury have been shown to accumulate primarily in the cortex and outer stripe of the outer medulla (Friberg et al., 1957; Bergstrand et al., 1959; Berlin, 1963; Berlin and Ullberg, 1963a,b; Taugner et al., 1966; Zalups and Barfuss, 1990; Zalups and Lash, 1990; Zalups, 1991a,b,c, 1993; Zalups and Cherian, 1992a,b). Until relatively recently, however, very little was known about which segments of the nephron take up and accumulate the various forms of mercury. This prompted numerous studies to determine where inorganic and organic forms of mercury are taken up and accumulated along the nephron. Histochemical and autoradiographic data from studies in mice and rats (Taugner et al., 1966; Hultman et al., 1985; Magos et al., 1985; Hultman and Enestrom, 1986, 1992; Rodier et al., 1988; Zalups, 1991a) and tubular microdissection data from studies in rats and rabbits (Zalups and Barfuss, 1990; Zalups, 1991b) indicate that the accumulation of inorganic mercury in the renal cortex and outer stripe of the outer medulla occurs mainly along the convoluted and straight segments of the proximal tubule. Deposits of mercury have also been localized in the renal proximal tubule of monkeys exposed to elemental mercury from dental amalgams (Danscher et al., 1990). It should be stressed, however, that although the segments of the proximal tubule appear to be the primary sites where mercuric ions are taken up and accumulated, there are currently insufficient data to exclude the possibility that other segments of the nephron and/or collecting duct may also, to a minor extent, take up, accumulate, and transport inorganic and/or organic forms of mercury.

It is interesting that deposits of presumed inorganic mercury have also been found along segments of proximal tubules in the kidneys of rats and mice treated with organic forms of mercury (Magos et al., 1985; Rodier et al., 1988). Additional findings indicate that a significant fraction of the mercury in the kidneys of animals exposed to methylmercury is in the inorganic form (Gage, 1964; Norseth and Clarkson, 1970a,b; Omata et al., 1980; Zalups et al., 1992), suggesting that organic mercury is oxidized to inorganic mercury before and/or after it enters the renal tubular epithelial cells. Furthermore, there is evidence that intracellular conversion of methylmercury to inorganic mercury can occur (Dunn and Clarkson, 1980). However, the mechanism for this conversion is currently unknown.

B. Mechanisms of Proximal Tubular Uptake and Transport of Mercury

Numerous theories and postulates regarding the mechanisms by which inorganic and organic forms of mercury gain entry into renal tubular epithelial cells have been put forth during the past two decades. In 1980, Madsen, and then later Zalups and Barfuss (1993b), put forth the hypothesis that a mechanism by which some mercuric ions gain entry into proximal tubular cells is through endocytosis of filtered mercury-albumin complexes. Albumin is by far the most abundant protein in plasma, and it has a free sulfhydryl group on a terminal cysteinyl residue (Brown and Shockley, 1982), to which mercuric ions can bind. Previous data indicate that the largest percentage of mercury in the plasma is bound to acid-precipitable proteins, such as albumin (Friedman, 1957; Mussini, 1958; Cember et al., 1968; Lau and Sarkar, 1979). Despite the fact that the sieving coefficient for albumin is low, significant amounts of protein, mainly albumin, are filtered during each day. Thus, the notion of albumin-mercury complexes being filtered at the glomerulus is a reasonable one. In fact, Madsen (1980) showed that when rats were made proteinuric by treatment with the proximal tubular toxicant gentamicin, inorganic mercury was excreted in the urine primarily as a conjugate of albumin. Assuming that the proteinuria (induced by gentamicin) was not due to increased glomerular permeability, these data suggest that a significant fraction of inorganic mercury that is filtered into the proximal tubular lumen is bound to albumin. Zalups and Barfuss (1993b) attempted to implicate a mercuric conjugate of albumin in the luminal uptake of inorganic mercury by simultaneously evaluating the renal disposition of inorganic mercury and albumin after administering mercuric conjugates of albumin containing both ^{125}I-albumin and $^{203}Hg^{2+}$. Although their data provided some interesting

new insights, there was insufficient evidence to implicate the transport of a mercuric conjugate of albumin as a primary mechanism involved in the luminal uptake of inorganic mercury. Conversely, there were insufficient data to exclude endocytosis of a mercuric conjugate of albumin as a minor mechanism.

A series of recent studies have provided much more definitive evidence on the mechanisms involved in the proximal tubular uptake of mercury. Data from these studies indicate that there are at least two distinct primary mechanisms involved in the uptake of mercuric ions by proximal tubular epithelial cells. One of the mechanisms is localized at the luminal membrane (Zalups et al., 1991, 1998; Zalups and Barfuss, 1993a, 1998b; Zalups, 1995, 1997, 1998b,c; Zalups and Minor, 1995; Zalups and Lash, 1997a) and the other is localized at the basolateral membrane (Zalups and Barfuss, 1993a, 1995a, 1998b; Zalups, 1995, 1997, 1998b; Zalups and Minor, 1995; Zalups and Lash, 1997a).

C. Mechanisms of Luminal Uptake of Mercury

1. Role of γ-Glutamyltransferase. There is a strong body of evidence linking the luminal uptake of inorganic mercury and, to a lesser extent, organic forms of mercury to the activity of γ-glutamyltransferase (γ-GT). In the kidney, this enzyme is localized predominantly in the luminal (brush-border) membrane of proximal tubular epithelial cells. The function of the enzyme is to cleave the γ-glutamylcysteine bond in molecules of glutathione (which are present in the proximal tubular lumen). Much of the evidence implicating the activity of the enzyme in the renal tubular uptake of mercury comes from in vivo experiments in which inhibition of renal (and hepatic) γ-GT, by pretreatment with L-(α*S*,5*S*)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (acivicin), has been shown to have profound effects on the renal disposition of administered mercury. More specifically, pretreatment with acivicin has been shown to cause significant decreases in the renal uptake and/or accumulation of mercury and significant increases in the urinary excretion of mercury in mice (Tanaka et al., 1990; Tanaka-Kagawa et al., 1993) and rats (Berndt et al., 1985; de Ceaurriz et al., 1994; Zalups, 1995) treated with inorganic mercury or in mice administered methylmercury (Tanaka-Kagawa et al., 1993) or exposed to mercury vapor (Kim et al., 1995). Enhanced urinary excretion of glutathione has also been documented in acivicin-pretreated rats that were subsequently injected with inorganic mercury (Berndt et al., 1985). Cannon et al. (1998a, 2000) recently provided direct evidence, from isolated perfused S2 segments of the rabbit proximal tubule, that inhibition of γ-GT (by the direct application of acivicin to the luminal plasma membrane) causes significant reductions in the luminal uptake (disappearance flux, J_D) and cellular accumulation of mercuric ions when they are in the form of mercuric conjugates of glutathione. Collectively, the in vivo and in vitro data described earlier indicate strongly that a significant fraction of the mercuric ions taken up by proximal tubular epithelial cells is accomplished by a luminal absorptive mechanism dependent on the actions of γ-GT.

2. Presence and Formation of Mercuric Conjugates in Proximal Tubular Lumen. A major implication of the data obtained during in vivo inhibition of γ-GT is that some pool of mercuric ions present in the lumen of the proximal tubule exists in the form of a mercuric *S*-conjugate of glutathione before being taken up. Although it is not known exactly where these mercuric conjugates of glutathione are formed before arriving in the lumen of the proximal tubule, one must consider the possibility that some of them are formed outside the kidneys and then enter into the lumen of the proximal tubule via glomerular filtration. There are a few reasons to suspect that this may occur. First, the formation of mercuric conjugates of glutathione in the plasma (after exposure to mercuric compounds) is theoretically possible because the concentration of this thiol-containing molecule in plasma (of rats) has been estimated to be approximately 10 μM (Lash and Jones, 1985a), which provides a sufficiently large pool of glutathione to form conjugates with mercuric ions in plasma. Second, the liver is a major source for glutathione in the body, and mercuric conjugates of glutathione have been shown to form in hepatocytes. Once formed, these conjugates may enter into systemic circulation along with glutathione, where they can then be delivered to the kidneys. Third, the size and shape of these conjugates are such that they can, and should, pass through the glomerular filtration barrier unimpeded.

One must also consider that a significant fraction of the pool of luminal mercuric conjugates of glutathione is actually formed in the lumen of the proximal tubule via mechanisms of thiol competition. In support of this notion are recent data demonstrating that approximately 75% of the glutathione synthesized de novo in pars recta segments of proximal tubules is secreted into the tubular lumen (Parks et al., 1998), which could theoretically provide a sufficiently high concentration of glutathione in the luminal compartment for thiol competition to occur.

Another possibility is that mercuric conjugates of glutathione are actually secreted into the lumen from within proximal tubular epithelial cells (after being formed intracellularly). There are data from mice that tend to support this hypothesis (Tanaka-Kagawa et al., 1993). The recent localization of the multiple-drug resistance glycoprotein MRP2 in the kidneys also tends to support the possibility of luminal secretion of mercuric *S*-conjugates of glutathione. This protein has been localized in the brush-border membrane of the epithelial cells lining the S1, S2, and S3 segments of the proximal tubule of the rat (Schaub et al., 1997) and the luminal plasma membrane of human proximal tubular epithelial

cells (Schaub et al., 1999). MRP2 is one of the ATP-binding cassette transport proteins, which has been shown recently to be involved in the intracellular to extracellular transport of glutathione *S*-conjugates at the canalicular membrane of hepatocytes (Keppler et al., 1998). Based on what is currently known about the cellular location and function of MRP2, it seems reasonable to hypothesize that intracellular mercuric *S*-conjugates of glutathione are also transported (in a secretory manner) by this protein in both hepatocytes and proximal tubular epithelial cells.

3. Cleavage Products of Mercuric Conjugates of Glutathione as Transportable Forms of Mercury at Luminal Plasma Membrane. Considering that luminal uptake of mercuric ions by proximal tubular cells is linked to the activity of γ-GT and the presence of mercuric *S*-conjugates of glutathione in the tubular lumen, the actual luminal uptake of mercuric ions would appear to involve the transport of some product formed by the actions of the γ-GT. One such product might be a mercuric conjugate of cysteinylglycine, which could be transported potentially by one of the small peptide transport systems in the luminal plasma membrane (Silbernagl, 1992). However, because of the high level of activity of luminal membrane dehydropeptidases (e.g., cysteinylglycinase), one would predict that if there is transport of this mercuric conjugate along the proximal tubule in vivo, the rate of transport would be very low. Based on the high activities of both γ-GT and cysteinylglycinase, it is most likely that the actual, or primary, species of mercury transported at the luminal membrane is a mercuric conjugate of L-cysteine, via one or more of the amino acid transport systems. It should be stressed that there is in vitro evidence indicating that sequential enzymatic degradation of glutathione to cysteinylglycine, and then to cysteine, is possible while a mercuric ion remains bound to the cysteinyl residue (at the site of the —SH group) of the molecules of glutathione that are being degraded (Naganuma et al. 1988).

4. Role of Cysteinylglycinase. Potential luminal transport of mercuric conjugates of cysteinylglycine was investigated recently in isolated perfused S2 segments of the rabbit proximal tubule by Cannon et al. (1998a, 2000). They demonstrated that near-complete inhibition of cysteinylglycinase, with the dehydropeptidase-1 inhibitor cilastatin, caused significant reductions in the luminal uptake of inorganic mercury when it was in the form of a mercuric *S*-conjugate of cysteinylglycine. These findings support the hypothesis that when inorganic mercury is conjugated to cysteinylglycine, much of the luminal absorption of mercury is linked to the actions of the dehydropeptidase-1 (cysteinylglycinase) that cleaves the peptide bond in molecules of cysteinylglycine. Cannon et al. (2000) discovered, however, that inhibition of luminal dehydropeptidases did not completely prevent the luminal uptake of mercury when it was in the form of a mercuric conjugate of cysteinylglycine. These findings tend to indicate that at least in isolated perfused proximal tubular segments, some level of transport of mercuric conjugates of cysteinylglycine may actually occur at the luminal membrane while luminal dehydropeptidases are inhibited. However, before one can make any definitive conclusions about potential transport of mercuric conjugates of cysteinylglycine in the proximal tubule in vivo, one needs to consider additional factors, such as potential heterogeneity in the handling of glutathione, cysteinylglycine, and mercuric conjugates of glutathione and cysteinylglycine along the entire proximal tubule. In fact, there are recent findings indicating there is significant heterogeneity in the synthesis, secretion, and/or transport of glutathione along the length of the rabbit proximal tubule (Parks et al., 1998, 2000).

5. Mercuric Conjugates of Cysteine as Primary Transportable Form of Mercury at Luminal Plasma Membrane. Numerous sets of recent findings indicate that mercuric conjugates of cysteine, such as the dicysteinylmercury complex, are likely the primary species of inorganic mercury transported at the luminal membrane of proximal tubular cells. For example, there are in vivo data showing that the renal uptake and accumulation of inorganic mercury (Zalups and Barfuss, 1995b, 1998b) and the level of renal tubular injury induced by inorganic mercury (Zalups and Barfuss, 1996b) were increased in animals when the inorganic mercury was administered as a mercuric conjugate of cysteine. In addition, there are in vitro data showing that mercuric ions gained entry into brush-border membrane vesicles far more readily when they were in the form of mercuric conjugates of cysteine than when they were in the form of mercuric conjugates of glutathione or even mercuric chloride (Zalups and Lash, 1997a). By far, the most convincing evidence for the luminal transport of a mercuric conjugate of cysteine comes from the isolated perfused tubule studies of Cannon et al. (1998a,b, 1999, 2000). These investigators demonstrated that the rates of luminal uptake (disappearance flux) of mercuric ions in isolated perfused proximal tubular segments were approximately 2-fold or more greater when mercuric conjugates of cysteine (103 ± 4 fmol/min/mm tubule) were present in the luminal compartment than when mercuric conjugates of either glutathione (39 ± 1 fmol/min/mm tubule) or cysteinylglycine (53 ± 3 fmol/min/mm tubule) were present in the lumen. Their findings also show that mercuric conjugates of cysteine, presumably in the form of a single mercuric ion bonded to the sulfur atoms of two molecules of cysteine (in a linear II coordinate covalent complex), are taken up at the luminal membrane of proximal tubular cells by known amino acid transporters (Cannon et al., 1999). These investigators also provide data indicating that the luminal uptake of these mercuric conjugates involves at least two separate amino acid transport systems, with one being sodium-dependent and the other being sodi-

um-independent. Another set of their data indicates that one or more of the same transport systems involved in the luminal uptake of the amino acid cystine may be involved in the luminal uptake of mercuric conjugates of cysteine (Cannon et al., 2000). These data show that the addition of 3 mM L-lysine to a perfusate containing 20 μM inorganic mercury and 80 μM cysteine caused an approximate 50% reduction in the net rate of luminal uptake of inorganic mercury in isolated perfused S2 segments of the rabbit proximal tubule. To put these findings into context, Schafer and Watkins (1984) had established previously in isolated perfused S2 segments that L-lysine (3 mM) inhibits the luminal uptake of cystine (300 μM) by approximately 50%. Their findings suggest that some component of the luminal absorption of cystine occurs through a transporter shared by the dibasic amino acid lysine. Overall, it appears that some fractions of the luminal uptake of both cystine and dicysteinylmercury occur via the same transport system. A diagrammatic summary of the known and putative mechanisms involved in the luminal transport of inorganic mercury is presented in Figs. 1 and 2.

6. Role of Molecular Homology. Based on experimental findings of Cannon et al. (2000) and Schafer and Watkins (1984) and the similarity in structure of cystine and the dicysteinylmercury complex (Fig. 3), researchers at the laboratories of Zalups and Barfuss hypothesized recently that some component of the absorptive luminal

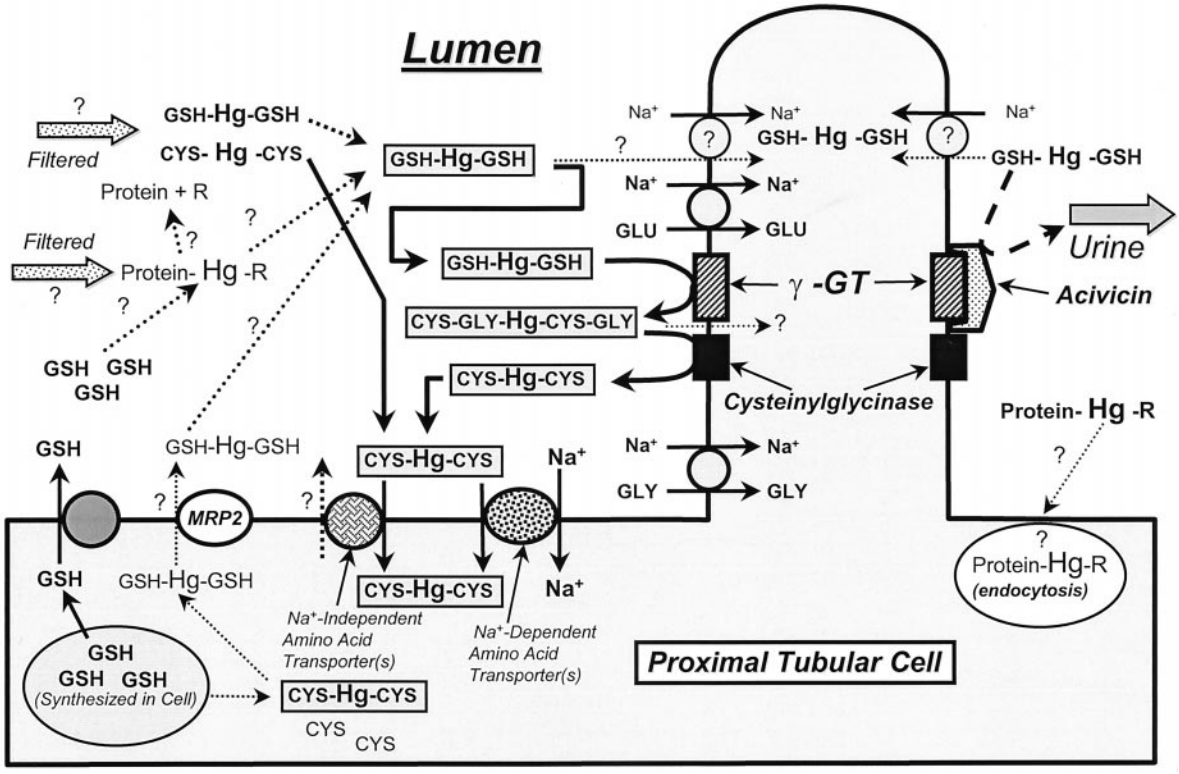

FIG. 1. Diagrammatic representation of mechanisms involved in the luminal uptake of inorganic mercury in proximal tubular epithelial cells. The presented scheme is premised on having mercuric conjugates of glutathione (GSH-Hg-GSH) and cysteine (CYS-Hg-CYS) present in the proximal tubular lumen. At present, it is not clear where these conjugates form, but filtration, secretion, and/or formation in the tubular lumen all must be considered. Experimental evidence indicates that the primary mechanism involved in the luminal uptake of inorganic mercury along proximal tubular segments involves the actions of the brush-border enzyme, γ-GT. When there is near-complete inhibition of the enzyme (with acivicin), there are significant decreases in the luminal uptake of inorganic mercury and enhanced urinary excretion of mercury and glutathione (GSH). According to the scheme presented, the mechanism of action of the γ-glutamyltranspeptidase in the luminal uptake of mercury involves the catalytic cleavage of the γ-glutamylcysteine bond on molecules of GSH bonded (via the sulfur atom of the cysteinyl residue) to mercuric ions. There also is the possibility that mixed mercuric conjugates may be present in the tubular lumen, but these have not been included in this figure. According to the presented scheme, after γ-GT has cleaved the γ-glutamylcysteine bond on molecules of GSH bonded to a mercuric ion, the resulting mercuric conjugate of cysteinylglycine can potentially enter two pathways. The most likely pathway involves the catalytic cleavage of the cysteinylglycinyl bond on molecules of cysteinylglycine bonded to a mercuric ion by the dehydropeptidase-1 cysteinylglycinase located on the luminal membrane. The second pathway might involve the transport of a mercuric *S*-conjugate of cysteinylglycine into the proximal tubular cell by one of the sodium-dependent peptide transport systems. This pathway is shown as a dotted line attached to an arrowhead, just above the depiction of cysteinylglycinase. Due to the abundance of peptidase activity on the luminal membrane of proximal tubular epithelial cells, it seems unlikely, at present, that any appreciable amount of transport of a mercuric conjugate of cysteinylglycine would occur under normal circumstances. After the actions of cysteinylglycinase, a mercuric conjugate of cysteine remains, presumably as dicysteinylmercury (CYS-Hg-CYS). This resulting mercuric *S*-conjugate of cysteine then appears to enter proximal tubular epithelial cells via amino transporters in the luminal plasma membrane. Current evidence indicates that there are both sodium-dependent and sodium-independent amino transport systems involved. One cannot exclude, however, the possibility that some type of a mercuric conjugate of GSH is also taken up intact by one of the luminal transport systems. This is shown using a dotted line attached to an arrowhead near the top of the figure. This scheme also shows the potential for endocytosis of a mercuric conjugate of albumin. Moreover, the scheme shows that the GSH secreted into the tubular lumen of proximal tubular segments may preferentially compete for the mercuric ions carried into the tubular lumen by albumin or other plasma proteins. Finally, this scheme shows that mercuric conjugates of cysteine can be filtered into the tubular lumen, bypass the actions of γ-GT and cysteinylglycinase, and enter the proximal tubular cells via one of the amino acid transporters.

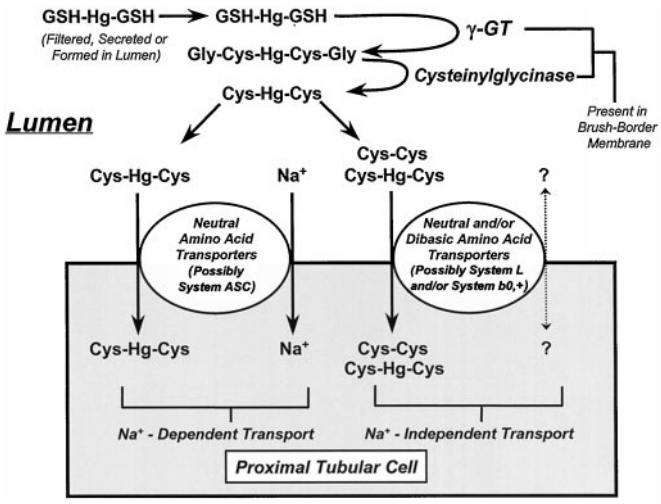

FIG. 2. This figure provides a few details on the potential mechanisms involved in the luminal uptake of the mercuric conjugate of cysteine, dicysteinylmercury (Cys-Hg-Cys), in proximal tubular segments. At least two amino acid transporters appear to be involved. One of these is a sodium-dependent transporter, and at least one of them is a sodium-independent transporter. A likely candidate for the sodium-dependent transporter involved in the uptake of dicysteinylmercury is System ASC. Among the transporters that are sodium-independent, System L or b0,+ are potential candidates. One of the current hypotheses regarding the mechanism by which some dicysteinylmercury gains entry into the proximal tubular epithelial cells is by a mechanism of molecular homology, which some refer to as "mimicry." The hypothesis states that the dicysteinylmercury complex is functionally homologous to the amino acid cystine and enters through one the transport systems involved in the absorption of cystine. Current molecular biological evidence from studies on the gene responsible for cystinuria indicate that System b0,+ is one transport system involved in the luminal transport of cystine. However, there is controversy on what other systems might be involved in the uptake of cystine along the various segments of the proximal tubule. Data from Cannon et al. (1999a) also tend to implicate System L in the luminal uptake of dicysteinylmercury.

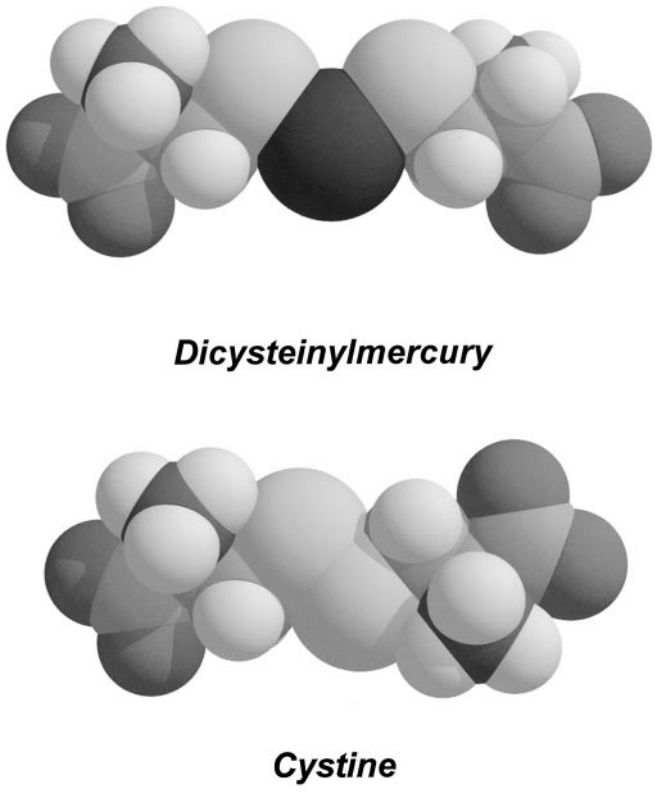

FIG. 3. A three-dimensional space-fill rendering of the molecule cystine and the complex formed by two molecules of cysteine bonded to an inorganic mercuric ion. This figure shows some of the homology that exists between these two molecules. A current hypothesis expounded to explain a significant component of the luminal uptake of dicysteinylmercury (along the proximal tubule) is one involving molecular homology. The dicysteinylmercury complex is thought to "mimic" or be functionally homologous to the amino acid cystine at the site of the transporters involved in the luminal uptake of cystine.

transport of dicysteinylmercury occurs by a mechanism involving molecular homology (or "mimicry"). They postulate that dicysteinylmercury may act as a molecular homolog, or "mimic," of the amino acid cystine at the site of one or more transporter responsible for the luminal uptake of cystine (Cannon et al., 2000).

Molecular homology, or what some refer to as molecular mimicry, is not a novel concept. In 1993, Clarkson discussed the concept that mercury, and other metals, form complexes with biological molecules that mimic structurally endogenous molecules. For example, the complex formed between methylmercury and cysteine is thought to "mimic" the amino acid methionine, as a means to gain entry into the central nervous system via specific amino acid transporters. Evidence supporting this hypothesis comes from studies on the uptake and/or transport of methylmercury by astrocytes (Aschner et al., 1990) and the endothelial cells lining the blood-brain barrier (Aschner and Clarkson, 1989, Kerper et al., 1992). Another potential transportable molecular homolog may occur when inorganic mercury or methylmercury binds to glutathione. The complex formed when two molecules of glutathione bind to a single mercuric ion may also prove to be a functional molecular homolog of glutathione disulfide. The implication of a dicysteinylmercury complex being homologous to, or "mimicking," the amino acid cystine does, however, appear to be a novel addition to the purported species of molecules that are involved in mimicry during the process of transporting a toxic metal into an epithelial cell.

D. Mechanisms of Basolateral Uptake of Mercury

1. Role of Organic Anion Transport System. In addition to the large body of evidence indicating that mercuric ions are taken up at the luminal membrane of proximal tubular cells, there is substantial evidence indicating that mercuric ions are also taken up at the basolateral membrane of these cells. Approximately 40% of the dose of inorganic mercury is normally taken by the total renal mass of rats during the initial hour after the i.v. injection of a nontoxic dose of mercuric chloride (Zalups and Diamond, 1987a; Zalups and Lash, 1994; Zalups and Barfuss, 1995a, 1998a,b; Zalups, 1996, 1997). Current evidence indicates that approximately 40 to 60% of this renal burden of mercury can be attributed to a basolateral mechanism (Zalups, 1995, 1997, 1998b,c; Zalups and Barfuss, 1995, 1998a,b; Zalups and Minor, 1995). It should be stressed that this applies only

to doses of inorganic mercury that are non-nephrotoxic. Under conditions where the dose is increased to levels that induce renal tubular injury, the percentage of the dose found in the kidneys (at various times after exposure) decreases. This is due in part to necrosis of tubular epithelial cells and the subsequent release and excretion of cytosolic mercury. (Zalups and Diamond, 1987b; Zalups et al., 1988).

One of the first lines of substantial evidence implicating a basolateral mechanism in the renal tubular uptake of inorganic mercury comes from a recent study by Zalups and Minor (1995). In this study, the uptake and disposition of administered inorganic mercury were evaluated in rats in which glomerular filtration had been reduced to negligible levels in one or both kidneys through pretreatment with mannitol in combination with ureteral ligation (Zalups and Minor, 1995). It was demonstrated that induction of "stop-flow" conditions by these pretreatments caused an approximately 40% decrease in the net uptake and accumulation of inorganic mercury during the initial 1 h after the administration of a 0.5 μmol/kg i.v. dose of mercuric chloride. These findings indicate that a major fraction of the renal tubular uptake of inorganic mercury occurred via a basolateral mechanism. They also demonstrated that pretreatment with *para*-aminohippurate, which is a specific competitive substrate for the renal organic anion transporter (Shimomura et al., 1981; Ferrier et al., 1983; Ullrich et al. 1987a,b); Pritchard, 1988; Roch-Ramel et al., 1992), caused significant reductions in the acute renal tubular uptake and accumulation of inorganic mercury in normal animals and in animals that had one or both ureters ligated. In fact, the combination of ureteral ligation and pretreatment with *para*-aminohippurate caused an approximately 85% reduction in the net uptake and accumulation of inorganic mercury during the first hour after the injection of mercuric chloride. These findings suggest that the majority of the basolateral uptake of inorganic mercury was being inhibited by *para*-aminohippurate, which implicates the organic anion transporter as the primary mechanism in the basolateral uptake of inorganic mercury. Data from other recent studies have confirmed that basolateral uptake of inorganic mercury does occur in the kidney and that the primary mechanism involved is linked to the activity of the organic anion transport system (Zalups and Lash, 1994; Zalups, 1995, 1997, 1998a,b; Zalups and Barfuss, 1995a, 1998a,b; Zalups et al., 1998).

There also are data implicating the activity of the organic anion transporter in the basolateral uptake of organic mercuric compounds. These data show that the renal uptake and/or accumulation (Tanaka et al., 1992) and toxicity (Ban and de Ceaurriz, 1988) of methylmercury are reduced significantly in mice pretreated with probenecid, which is another competitive substrate and inhibitor of the organic anion transporter in renal proximal tubules (Shimomura et al., 1981; Roch-Ramel et al., 1992).

2. Role of Dicarboxylate Transporter. In an early study, Clarkson and Magos (1967) demonstrated that pretreatment with the dicarboxylate maleate caused dose-dependent reductions in the net renal accumulation of inorganic mercury when it was given as a cysteine-mercury complex (100 μg Hg/kg). Unfortunately, it is not clear from this study whether the changes in the renal disposition of mercury were due to the inhibitory effects of maleate on renal cellular metabolism (Rogulski and Angielski, 1963) or whether they were due to direct effects at the site of a transporter of mercury. Interestingly, they found that fumarate (an isomer of maleate) did not have the same effects as maleate, which suggests isomer specificity.

More recently, Zalups and Barfuss (1998b) demonstrated that pretreatment with small (four- to six-carbon) aliphatic dicarboxylates, such as succinate, glutarate, or adipate (but not malonate), inhibited the renal (basolateral) uptake of i.v. administered inorganic mercury in a dose-dependent manner in both normal rats and in rats that had their ureters ligated. Putative mechanisms for the inhibitory effects of certain dicarboxylates on the renal tubular uptake, transport, and accumulation of inorganic mercury have been hypothesized by Zalups and Barfuss (1998b). Some of the details of these hypotheses are provided here.

Current evidence indicates that the organic anion transporter is driven by an organic anion/dicarboxylic acid (dicarboxylate) exchange (reviewed by Pritchard and Miller, 1993, and Dantzler, 1996). It appears that intracellular generation of α-ketoglutarate (from normal metabolic processes) contributes to the creation of an intracellular chemical gradient favoring the movement of this dicarboxylate out of the cell. When the gradient becomes sufficiently great, α-ketoglutarate is transported out of proximal tubular cells at the basolateral membrane via exchange with organic anions at the site of the organic anion exchanger. There is evidence indicating that a significant fraction of the α-ketoglutarate (and other dicarboxylic acids) that exits proximal tubular cells at the organic exchanger enters back into the cells across the basolateral membrane via a sodium-dicarboxylic acid cotransporter (Pritchard, 1988). This cotransport system is driven by the sodium-gradient generated by Na^+,K^+-stimulated ATPase. Although it is not exactly clear via which mechanisms succinate, glutarate, or adipate inhibits the renal tubular uptake of inorganic mercury, it seems likely that an excess of any of these dicarboxylates in the extracellular compartment creates competition for the sodium-dependent entry of α-ketoglutarate at the site of the dicarboxylic acid cotransporter. Reduction in the basolateral uptake of α-ketoglutarate would likely cause a decrease in the intracellular concentration of this dicarboxylate. This in turn would decrease the chemical gradient favoring the

movement of α-ketoglutarate out of the proximal tubular epithelial cell in exchange for the uptake of an organic anion from the plasma. The net result would be a decreased rate of uptake of organic anions (and presumably mercuric conjugates of cysteine and/or glutathione) that are transported at this site. Because dicarboxylates are themselves organic anions, an excess of these molecules in the extracellular fluid likely also creates direct competition with whatever form of mercury that is putatively transported by the organic anion transporter and, thus, contributes to a decreased rate of uptake of mercury at the basolateral membrane. There is evidence that both adipate and glutarate, but not succinate or malonate, can compete with α-ketoglutarate at the site of the organic anion transport system (Ullrich et al., 1987; Pritchard, 1988; Pritchard and Miller, 1993). Figure 4 presents some of the mechanisms involved in the basolateral uptake of inorganic mercury in proximal tubular cells.

3. Possible Ligands and Conjugates Involved in Basolateral Uptake of Mercury. As mentioned earlier, the majority of the mercury that is present in plasma is bound to albumin and other large proteins. It is quite certain that the organic anion transport system does not transport mercuric conjugates of proteins into proximal tubular epithelial cells. At present, it appears that mercuric conjugates of low-molecular-weight ligands are the most likely species of mercury taken up at the basolat-

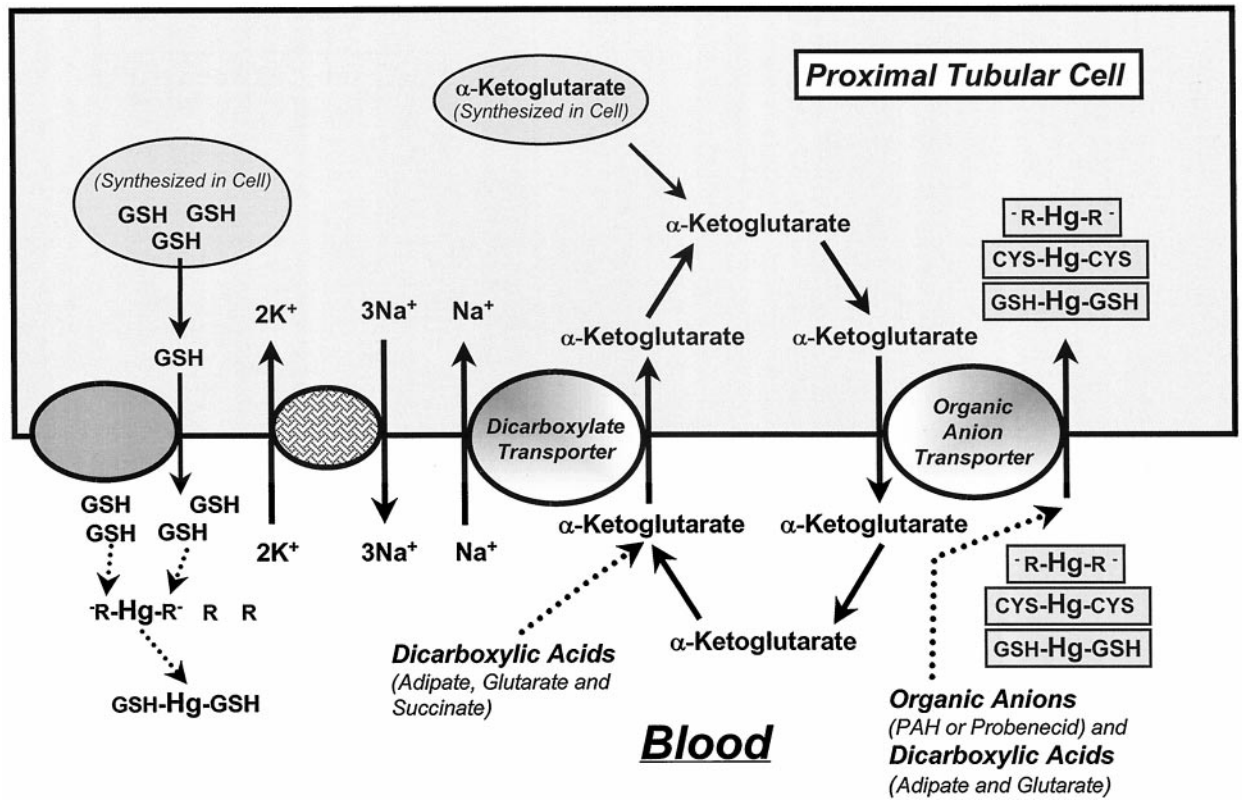

FIG. 4. Diagram outlining the putative roles of both the organic anion and dicarboxylic acid transport systems in the basolateral uptake of inorganic mercury along the proximal tubule. On the basis of the current line of evidence regarding the organic anion transport system, intracellular generation of α-ketoglutarate (as a result of normal metabolic processes) creates a chemical gradient facilitating the movement of this dicarboxylate out of the cell. When intracellular concentrations of α-ketoglutarate are sufficiently high, it exits proximal tubular cells at the basolateral membrane by exchanging with organic anions. After it is transported out of proximal tubular cells, α-ketoglutarate is taken back up into the cell across the basolateral membrane via a sym-port involving the cotransport of sodium. This sym-port is driven by the sodium-gradient generated by the Na^+,K^+-ATPase localized in the basolateral membrane. According to the scheme presented, inorganic mercury enters proximal tubular epithelial cells (presumably as a conjugate of glutathione (GSH) and/or cysteine (CYS)) via the organic anion transport system in exchange for intracellular α-ketoglutarate. The most likely species of inorganic mercury taken up at basolateral membrane by the organic anion exchanger include mercuric conjugates of GSH (GSH-Hg-GSH), CYS (CYS-Hg-CYS), and other small molecules possessing a negative charge ($^-$R-Hg-R$^-$), such as *N*-acetylcysteine. Support for this notion comes from the fact that the basolateral uptake of mercury can be inhibited by *para*-aminohippurate (PAH) and probenecid and dicarboxylic acids. The scheme presented also shows that succinate, glutarate, and adipate compete with α-ketoglutarate at the site of the dicarboxylic acid transporter. It appears that both glutarate and adipate, but not succinate, can influence the basolateral uptake of mercury by theoretically acting at two sites. Evidence indicates that these two dicarboxylic acids can compete for the uptake of substrates transported by the organic anion transporter and other dicarboxylic acids (e.g., α-ketoglutarate) transported by the sodium-dicarboxylic acid sym-port system. Succinate, on the other hand, appears to influence the basolateral uptake of mercury by competing only at the site of the dicarboxylic acid transporter. Preferential uptake of succinate over α-ketoglutarate would result in a decrease in the intracellular concentration of α-ketoglutarate, which would decrease the driving force behind the activity of the organic anion transporter. The scheme also shows the transport of newly synthesized GSH from within proximal tubular cells into the blood. There is evidence from isolated perfused proximal tubular segments demonstrating that approximately 25 to 30% of the GSH synthesized by proximal tubular epithelial is secreted into the basal compartment surrounding the tubule. This could provide a substantial pool of GSH at the basolateral membrane to interact with other molecules bonded to mercuric ions, resulting in the formation of mercuric conjugates of GSH, which could then be transported into the proximal tubular cells.

eral membrane by the organic anion transporter. Two of the conjugates that have been implicated in the basolateral transport of mercury are mercuric conjugates of glutathione and/or cysteine (Zalups, 1998b).

4. Mercuric Conjugates of Glutathione as Transportable Forms of Mercury at Basolateral Membrane. Molecules of glutathione have a net negative charge at physiological pH. Because of this charge and its size, glutathione has been postulated to be substrate at the site of the organic anion transporter. Support for this comes in part from the studies of Lash and Jones (1983, 1984), who demonstrated transport of glutathione (as an intact tripeptide) in basolateral membrane vesicles (isolated from the renal cortex of rats) via a mechanism that was sodium-dependent and that could be blocked by probenecid. They also demonstrated basolateral transport of certain organic *S*-conjugates of glutathione, such as *S*-(1,2-dichlorovinyl)glutathione, into proximal tubular epithelial cells by a probenecid-sensitive mechanism (Lash and Jones, 1985b).

Because both glutathione and certain *S*-conjugates of glutathione appear to be transported across the basolateral membrane of proximal tubular cells by the organic anion transport system, it seems plausible that mercuric *S*-conjugates of glutathione may also be transported across the basolateral membrane by this same transport system. There are some findings from a recent study, in which mercuric conjugates of glutathione were administered to rats that had undergone bilateral ureteral ligation, that support this contention (Zalups, 1998b). The data show that the basolateral uptake of inorganic mercury was greater when it was administered in the form of a mercuric conjugate of glutathione than when it was administered as mercuric chloride.

5. Mercuric Conjugates of Cysteine as Transportable Forms of Mercury at Basolateral Membrane. Despite the fact that cysteine has a net neutral charge at physiological pH, it has become highly relevant to consider that inorganic or organic mercuric conjugates of cysteine are transportable species at the site of the organic anion transporter. The relevance for this consideration comes from studies in which organic *S*-conjugates of cysteine have been shown to be taken up at the basolateral membrane of proximal tubular cells by a mechanism consistent with the activity of the organic anion transporter. For example, Lash and Anders (1989) demonstrated that organic *S*-conjugates of cysteine [e.g., *S*-(1,2-dichlorovinyl)-L-cysteine] were taken up by isolated proximal tubular epithelial cells from rats by a sodium-dependent and probenecid- and *para*-aminohippurate-sensitive transport system. More recently, Dantzler et al. (1995), using isolated proximal tubules from rabbits, also demonstrated that certain organic *S*-conjugates of cysteine were taken up at the basolateral membrane by a probenecid- and *p*-aminohippurate-sensitive transport mechanism.

Based on these aforementioned findings, it seems logical to hypothesize that mercuric conjugates of cysteine may also be transported into proximal tubular epithelial cells at the basolateral membrane by the organic anion transport system. Several sets of recent data tend to support this hypothesis. For example, one set of data shows that rates of association and transport of inorganic mercury in basolateral membrane vesicles (isolated from the kidneys of rats) tends to be greater when the vesicles are exposed to mercuric conjugates of cysteine than when they are exposed to mercuric chloride (Zalups and Lash, 1997a). Additional support for this hypothesis comes indirectly from a recent in vivo study (Zalups, 1998b). First, the data from this study show that bilateral ureteral ligation caused an approximately one-half reduction in the net renal accumulation of mercury in control rats treated with a low 0.5-μmol/kg dose of mercuric chloride and in the rats coadministered this dose of inorganic mercury with a 4-fold greater (2.0 μmol/kg) amount of L-cysteine. More importantly, the findings also show that the net renal accumulation of mercury was greater in the animals treated with inorganic mercury plus cysteine than in the animals treated with mercuric chloride, whereas the relative intrarenal distribution of mercury was similar in both groups of rats. Second, pretreatment with *para*-aminohippurate was shown to cause a significant decrease in the renal uptake of mercury in the rats that had their ureters ligated and that were administered inorganic mercury plus cysteine (Zalups, 1998c). The most reasonable explanation for these findings is that by injecting mercuric conjugates of cysteine in animals that have had their ureters ligated, more of these conjugates than are formed normally when inorganic mercury is administered as mercuric chloride are made available at the site of the organic anion transporter (and possibly other basolateral transporters, such as basolateral amino acid transporters) to promote the uptake of mercury.

6. Other Mercuric Conjugates as Transportable Forms of Mercury at Basolateral Membrane. Although current experimental evidence tends to point to mercuric conjugates of cysteine and glutathione being primarily involved in the luminal and basolateral uptake of inorganic mercury along the proximal tubule (after exposure to mercuric chloride), it is clear that other thiols, especially homologues of cysteine, such as homocysteine and *N*-acetylcysteine, can significantly influence the manner in which inorganic mercury is being handled in the kidneys (Zalups, 1998c; Zalups and Barfuss, 1998b). This point is exemplified in the recent studies of Zalups and Barfuss (1998b) and Zalups (1998c), who studied and compared in rats the mechanisms involved in the renal tubular uptake of inorganic mercury when it was coadministered with cysteine, homocysteine, or *N*-acetylcysteine. When inorganic mercury was administered with cysteine or as mercuric chloride, the levels of luminal and basolateral uptake of mercury in the kidneys

were similar. In contrast to this pattern of uptake, when inorganic mercury was administered with homocysteine, a much lower level of uptake of mercury occurred at the luminal membrane relative to that which occurred at the basolateral membrane. Even greater differences in the levels of luminal uptake versus basolateral uptake of mercury were detected when rats were treated with inorganic mercury and *N*-acetylcysteine. When inorganic mercury was administered with this negatively charged molecule, virtually all of the renal tubular uptake of mercury occurred at the basolateral membrane, and the majority of this uptake could be inhibited by pretreatment with *para*-aminohippurate. In fact, regardless of how inorganic mercury was administered, the majority of the basolateral uptake of mercury was inhibited by pretreatment with *para*-aminohippurate, which implicates the activity of the organic anion transport system in the basolateral uptake of inorganic mercury under all of the experimental conditions studied.

In addition to the high level of basolateral uptake of mercury in the kidneys of the animals treated with inorganic mercury and *N*-acetylcysteine, the amount of mercury excreted in 24 h was at least 45 to 50% greater than that in any of the other groups of rats. The overall findings from these rats indicate that the negative charge on *N*-acetylcysteine likely promotes the rapid transport of mercuric conjugates of *N*-acetylcysteine into proximal tubular cells at the site of the organic anion transporter, whereas it prevents or impedes the uptake of these mercuric conjugates at the luminal plasma membrane, which promotes the urinary excretion of mercury.

E. Role of Liver in Renal Tubular Uptake of Mercury

It appears that some aspects of hepatic function play a role in at least a component of the renal uptake and transport of mercury. Evidence for this hypothesis comes from recent dispositional studies. In one study, specific depletion of hepatic glutathione with 1,2-dichloro-4-nitrobenzene before the administration of inorganic mercury was shown to cause a significant diminution in the renal uptake and/or accumulation of inorganic mercury in mice (Tanaka et al., 1990). In other studies, it has been demonstrated that biliary ligation or cannulation before the administration of inorganic mercury caused a decrease in the renal tubular uptake and accumulation of inorganic mercury in rats (Zalups and Barfuss, 1996a, Zalups, 1998a; Zalups et al., 1999a,b,c). Taken together, these findings indicate that some aspects of hepatic function are linked to a component in the renal tubular uptake and/or accumulation of inorganic mercury. Hepatic synthesis and secretion of glutathione represent a possible candidate. Additional studies are necessary to better determine the role of the liver in the renal tubular uptake of mercury.

F. Intracellular Distribution of Mercury

Once inorganic mercuric ions gain entry in proximal tubular cells, it appears that they distribute throughout all intracellular pools (Madsen, 1980; Omata et al., 1980; Baggett and Berndt, 1985; Houser and Berndt, 1988). Cellular fractionation studies using the renal cortex from rats treated acutely or chronically with mercuric chloride indicate that mercury distributes in nuclear, lysosomal, mitochondrial, brush-border, and supernatant fractions, with the nuclear fraction containing the greatest amount of mercury among the organelle fractions (Madsen, 1980; Madsen and Hansen, 1980). Similar findings have also been obtained in other studies using homogenates of the renal cortex from normal and uninephrectomized rats treated with mercuric chloride (Baggett and Berndt, 1985; Houser and Berndt, 1988). In these studies, however, the cytosolic fraction was found to contain the greatest content of mercury.

Interestingly, the relative specific content of mercury was shown to increase to the greatest extent in the lysosomal fraction when rats were made proteinuric with an aminoglycoside (Madsen, 1980) or when rats were treated chronically with mercuric chloride (Madsen and Hansen, 1980). Increases in the lysosomal content of mercury may reflect the fusion of primary lysosomes with endocytotic or cytosolic vesicles containing complexes of inorganic mercury bound to proteins.

III. Urinary Excretion of Mercury

Urinary and fecal excretion of mercury are the principal means by which humans and other mammals eliminate the different forms of mercury from the body. Under most circumstances, a greater fraction of a dose of mercury is excreted in the feces than in the urine early after exposure (Rothstein and Hayes, 1960; Magos and Clarkson, 1977; Zalups et al., 1987, 1988, 1991a, 1992, 1993; World Health Organization, 1991; Zalups et al., 1987, 1988, 1991a, 1992, 1993; Zalups and Lash, 1994). In rats, it has been shown that more than twice as much inorganic mercury is excreted in the feces than in the urine during the initial days after exposure to a non-nephrotoxic dose of mercuric chloride (Rothstein and Hayes, 1960; Zalups et al., 1987, 1988; Zalups and Lash, 1994). Less than 10% of the administered dose is excreted in the urine during this time. In one study, rats injected i.v. with a non-nephrotoxic dose of mercuric chloride had excreted about 20% of the dose in the urine and 30% of the dose in the feces during the initial 54 days after injection (Rothstein and Hayes, 1960). The low level in the urinary excretion of mercury is due to two principal factors, the avid uptake of mercuric ions and the retention of accumulated mercuric ions, in proximal tubular segments.

After exposure to organic forms of mercury, even less mercury is excreted in the urine than after exposure to inorganic mercury. For example, it was demonstrated

recently that both normal and uninephrectomized rats excreted only about 3% of the dose of mercury in the urine by the end of the initial 7 days after the i.v. injection of a low dose (5 mg/kg) of methylmercury (Zalups et al., 1992). By contrast, more than 15% of the administered dose was excreted in the feces during the same period of time. In a recent study in which seven adult men received a tracer amount of ^{203}Hg-labeled methylmercury i.v., the cumulative fecal excretion of mercury over 70 days was much greater than the cumulative urinary excretion of mercury (Smith et al., 1994). More specifically, about 30% of the dose was excreted in the feces, whereas only about 4% of the dose was excreted in the urine.

Early reports (Mambourg and Raynaud, 1965; Vostal, 1966) had claimed that mercury appeared in the urine before inulin (which is filtered and not absorbed or secreted along the nephron). This was interpreted by some (Clarkson and Magos, 1967) to indicate that urinary mercury represented a pool of mercury that had been secreted from the blood into the tubular lumen by a transepithelial mechanism. This was a reasonable view considering there was a published report claiming that approximately 99% of the mercury in plasma was not filterable (Berlin and Gibson, 1963). Based on recent data, however, it appears that much more than 1% on the mercury in plasma is filtered into the proximal tubule lumen (Madsen, 1980; Zalups and Minor, 1995; Zalups, 1997, 1998b,c; Zalups and Barfuss, 1998a,b) and that the mechanisms involved in the urinary excretion of mercury are less clear than once thought.

It should be emphasized that although 95 to 99% (depending on animal species and experimental conditions) of the mercury in plasma is bound to albumin (and other plasma proteins), a significant fraction of albumin is filtered at the glomerulus. Thus, substantial amounts of mercury could theoretically gain access to the luminal compartment of proximal tubules by filtration of a mercury-albumin complex. There is some indirect in vivo evidence supporting this notion. Madsen (1980) demonstrated in rats made proteinuric by gentamicin (presumably by decreasing the absorptive capacity of the proximal tubular epithelium by cellular necrosis) that much of the administered mercury excreted in the urine was associated with albumin. A fundamental assumption in with these findings, however, is that the preponderance of the albumin associated with the mercury in the urine came from glomerular filtration rather than intercellular leak. In contrast to the findings of Madsen (1980), Clarkson and Magos (1967) found that about 70% of the mercury excreted in urine by rats treated with sodium maleate, subsequent to the exposure of inorganic mercury, was not bound to protein. This finding is actually not that surprising, because much of the mercury excreted in the urine probably originated from cellular stores, and thus was likely bound to low-molecular-weight thiols, such as glutathione.

Some insight into mechanisms involved in the urinary excretion of mercury has been gained through experimental maneuvers that cause the urinary excretion of mercury to increase. In most cases, the increased urinary excretion of mercury is associated with decreased luminal absorption of mercury and/or the luminal elimination or extraction of accumulated mercury along the proximal tubule (and/or other segments of the nephron). Some examples of these maneuvers are listed below.

In an early study by Clarkson and Magos (1967), pretreatment of female rats with sodium maleate, before the injection of a low 100 μg/kg dose of mercury in the form of mercuric chloride or a mercury-cysteine complex, was shown to cause the urinary excretion of mercury to increase and the renal accumulation of mercury to decrease. Sodium maleate was used because it caused "profound metabolic disturbances in renal cells." The authors also found that the administration of sodium maleate after treatment with mercury caused the renal content of mercury to decrease and the urinary excretion of mercury to increase.

As mentioned earlier, the urinary excretion of mercury also increases dramatically when renal γ-GT is inhibited before the administration of inorganic mercury (Berndt et al., 1985; Zalups, 1995; Zalups et al., 1999b,c). Much of the mercury excreted in urine after the inhibition of γ-GT appears to be associated with glutathione, which implicates the presence of mercuric conjugates of glutathione in the proximal tubular lumen (Baggett and Berndt, 1986). Current evidence indicates that the increased urinary excretion of mercury associated with the inhibition γ-GT is due mainly to decreased luminal absorption and transport of mercury along the proximal tubule (Berndt et al., 1985; Tanaka et al., 1990; Tanaka-Kagawa et al., 1993; de Ceaurriz et al., 1994; Kim et al., 1995; Zalups, 1995; Cannon et al., 2000).

When inorganic mercury is applied to the luminal membrane of proximal tubular epithelial cells as a mercuric conjugate of *N*-acetylcysteine (Zalups and Barfuss, 1998b), DMPS (Zalups et al., 1998), DMSA (Zalups, 1993c), or metallothionein (Zalups et al., 1993a), urinary excretion of mercury increases greatly due to the lack of luminal uptake of these mercuric conjugates. In general, it appears that when mercuric ions are bound to organic ligands possessing a net negative charge, the mercuric conjugates of these molecules are not taken up readily at the luminal membrane and in turn are excreted in the urine. When DMPS is administered after exposure to mercury, the urinary excretion of mercury also increases greatly (Zalups, 1993c). Recent evidence (obtained from isolated perfused proximal tubular segments) indicates that the increased urinary excretion of mercury that occurs under these conditions results from unidirectional extraction of mercury from within or on proximal tubular epithelial cells into the tubular lumen (Zalups et al., 1998). It is likely that increased urinary excretion of

mercury induced by treatment with DMSA (Zalups, 1993c) occurs by a similar mechanism.

After proximal tubular necrosis is induced by mercury or other agents (Clarkson and Magos, 1967; Magos and Stoychev, 1969; Trojanowska et al., 1971), the urinary excretion of mercury increases. This is due largely to mercury being released from, or not being absorbed by, necrotic or degenerating proximal tubular epithelial cells (Madsen, 1980; Zalups and Diamond, 1987b; Zalups et al., 1988). More studies are needed to better define the factors and mechanisms involved in the urinary excretion of mercury and mercury-containing compounds.

Despite all the studies that have been carried out to date, very little is really known about the mechanisms involved in the urinary excretion of inorganic and organic forms of mercury. The major questions that still need to be addressed include the following. 1) What are the magnitudes and rates at which mercury is filtered at the glomerulus? 2) To what extent is filtered mercury taken up by proximal tubular epithelial cells? Alternatively, to what extent is filtered mercury excreted in the urine? 3) What is the chemical form or forms of mercury excreted in the urine? 4) Is some of the mercury that is excreted in the urine added to the luminal fluid by a *trans*-epithelial secretory mechanism (as has been suggested previously by Clarkson and Magos, 1967; Foulkes, 1974; Zalups and Barfuss, 1993a, 1995a, 1998b; Zalups, 1995, 1997, 1998b; Zalups and Minor, 1995; Zalups and Lash, 1997a)?

IV. Molecular Interactions and Effects of Mercury in Renal Epithelial Cells

A. Effects of Mercury on Intracellular Thiol Metabolism

A major intracellular effect of mercury consists of the induction of and binding to metallothionein (Piotrowski et al., 1974). Metallothioneins are a group of small intracellular proteins with an approximate molecular weight of 6000 to 7000 Da. They contain numerous cysteinyl residues and have the capacity to bind various metals, including inorganic mercury, cadmium, zinc, copper, silver, and platinum. The administration of a single, daily, nontoxic dose of mercuric chloride over several days has been shown to cause a near doubling in the concentration of metallothionein in the renal cortex or outer stripe of the outer medulla in rats (Zalups and Cherian, 1992a). Induction of the synthesis of metallothionein in kidney has also been demonstrated in rats exposed to elemental mercury vapor over the course of several days (Cherian and Clarkson, 1976). The increase appears to be tissue-selective, as changes in hepatic metallothionein synthesis have not been demonstrated. Mercury vapor is converted into inorganic mercury, which is recovered predominantly (approximately 98%) in the kidney, suggesting that the induction of metallothionein in the kidneys after exposure to elemental mercury may actually be mediated by inorganic mercury. The induction of metallothioneins in the kidney by inorganic mercury likely involves increased transcription of metallothionein-1 (*MT-1)* and metallothionein-2 (*MT-2)* genes via the interaction of zinc-dependent metal transcription factors and *cis*-acting DNA elements termed metal-responsive elements resident in the promoter region of the metallothionein genes. Sebsequently, there is post-transcriptional control of translation of new mRNA into metallothionein I and II protein (Koropatnick and Zalups, 2000).

Some new insights into the relationships between the cellular content of mercury and the expression of metallothionein in both the kidneys and liver have been provided in a recent study by Zalups and Koropatnick (manuscript submitted). They obtained evidence that the retention of inorganic mercury by renal tubular epithelial cells is associated with the continual induction of metallothionein. More specifically, their data indicate that in rats administered a single 0.5 μmol/kg i.v. dose of mercuric chloride, the rate of transcription of *MT-1* and *MT-2* genes was as great at 2 weeks after treatment with mercury as it was 1 day after treatment. In addition, renal levels of metallothionein-1 and -2 protein remained elevated throughout the 2 weeks of study, during which the renal burden of mercury decreased by only about 26% and the cumulative urinary excretion of mercury was equal to about 24% of the dose of mercury. By contrast, they demonstrated that hepatic levels of mercury and metallothionein protein and rates of transcription of *MT-1* and *MT-2* genes decreased continually over the initial 2 weeks after treatment. It was also demonstrated that the rates of transcription of metallothionein genes in the liver correlated highly with the amount of metallothionein protein in the liver. However, in the kidneys, there was no correlation between the rates of transcription of metallothionein genes and metallothionein protein, which suggests that post-transcriptional events are involved in the expression of metallothionein protein in the kidneys after a single exposure to inorganic mercury.

Inorganic and organic forms of mercury also have a great influence on intracellular glutathione metabolism in the kidneys. These effects are observed acutely after short-term, single treatments and are concentration-dependent. Several sets of in vivo and in vitro data demonstrate increases in intracellular contents of glutathione in renal tubular epithelial cells after the administration of relatively low toxic or nontoxic doses of either methylmercury (Woods et al., 1992) or inorganic mercury (Fukino et al., 1986; Siegers et al., 1987; Zalups and Lash, 1990; Chung et al., 1982; Lash and Zalups, 1992, 1993). At higher doses of inorganic mercury, decreases in renal content of glutathione (which are often substantial) are observed (Fukino et al., 1984; Zalups and Lash, 1990; Lash and Zalups, 1992, 1993).

Dose-dependent effects of inorganic mercury on renal glutathione metabolism have been demonstrated in male Sprague-Dawley rats that received one of several nontoxic or nephrotoxic i.v. doses of mercuric chloride (Zalups and Lash, 1990). At the level of the whole kidney or in samples derived from the renal cortex or the outer stripe of the outer medulla, the nontoxic (0.5 μmol/kg) or the moderately nephrotoxic (2 μmol/kg) dose of mercuric chloride induced significant increases in the renal concentration of glutathione. This effect was most marked in the outer stripe of the outer medulla, where the concentration of glutathione increased by as much as 85%. The toxicological significance of this finding relates to the fact that the outer stripe of the outer medulla is one of the primary zones in which proximal tubular injury induced by mercury occurs. At the highest nephrotoxic dose of mercuric chloride (3 μmol/kg), the concentrations of glutathione in the renal cortex and outer stripe of the outer medulla were similar to those in controls (Zalups and Lash, 1990).

Because the cellular content of glutathione is under feedback control, the large increases in the renal content of glutathione observed after treatment with inorganic mercury suggest that subtoxic or moderately toxic doses of inorganic mercury induce the synthesis of glutathione via γ-glutamylcysteine synthetase (GCS), which is the rate-limiting enzyme involved in the biosynthesis of glutathione. Data from Lash and Zalups (1993) support this hypothesis. They found that the activity of γ-glutamylcysteine synthetase was increased in renal proximal tubular epithelial cells isolated from rats treated with inorganic mercury (relative to that in proximal tubular cells isolated from control rats). Further support for the hypothesis that mercuric ions induce GCS in renal epithelial cells comes from a study by Woods et al. (1992). They showed that the mRNA for γ-glutamylcysteine synthetase increased (by 4.4-fold) in the kidneys of male Fischer 344 rats treated with methylmercury hydroxide for 3 weeks. Thus, at non-nephrotoxic doses, both inorganic and organic forms of mercury appear to induce the synthesis of glutathione via the activity of GCS.

In addition to causing up-regulation of GCS, inorganic mercury also alters, in a dose-dependent manner, the activity of other glutathione-dependent enzymes. The effects of inorganic mercury on these enzymes differ depending on whether a nontoxic, a moderately toxic, or a highly toxic dose is administered. Nontoxic doses of mercuric chloride apparently cause increases in activities of glutathione disulfide reductase and glutathione peroxidase in isolated epithelial cells from both proximal tubular and distal tubular regions of the rat nephron (Lash and Zalups, 1993). In contrast, one group of investigators (Addya et al., 1984) observed marked decreases in the activities of renal glutathione disulfide reductase and glutathione peroxidase in male rats treated chronically (15 days) with a relatively high dose of mercuric chloride (5 mg $HgCl_2$/day per os). They also found apparent adaptive increases in catalase activity. Similarly, others have found significant decreases in the activity of glutathione disulfide reductase after the administration of highly nephrotoxic doses of mercuric chloride [10 μmol $HgCl_2$/kg s.c. (Chung et al., 1982); 15 μmol $HgCl_2$/kg s.c. (Fukino et al., 1984); 4 mg $HgCl_2$/kg s.c. (Gstraunthaler et al., 1983)]. Although two groups of investigators found small (20–35%), but statistically significant, decreases in the activity of glutathione peroxidase (Chung et al., 1982; Gstraunthaler et al., 1983), another group did not detect any change in the activity of this enzyme (Fukino et al., 1984). It is important to keep in mind, however, that it becomes nearly impossible to interpret in vivo data obtained from renal tissue in which there has been extensive cellular injury and death. When there is extensive renal tubular necrosis, decreases in the content of an enzyme or a molecule of interest (in samples of renal tissue) can be accounted for simply by the release and excretion of the cytoplasmic contents from dead epithelial cells.

B. Role of Lipid Peroxidation and Oxidative Stress in Mercury-Induced Renal Cellular Injury

Findings from several studies suggest that an important mechanism involved in renal cellular injury induced by either in vivo or in vitro exposure to inorganic or organic forms of mercury involves the induction of oxidative stress. The high affinity of mercuric ions for binding to thiols naturally suggests that the ensuing depletion of intracellular thiols (especially glutathione) either directly causes, or predisposes, proximal tubular cells to oxidative stress. Furthermore, other cellular antioxidants, including ascorbic acid and vitamin E, have been reported to be depleted in the kidneys of rats treated with mercuric chloride (Fukino et al., 1984). The activity of several antioxidant enzymes also appears to be markedly diminished after in vivo exposure of rats to nephrotoxic doses of mercuric chloride. For example, it has been reported that the administration of mercuric chloride to male Sprague-Dawley rats caused marked decreases in the activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione disulfide reductase in the renal cortex (Gstraunthaler et al., 1983).

Decreases in the activities of these protective enzymes would be expected to enhance the susceptibility of renal epithelial cells to oxidative injury. There has been some disagreement as to whether mercury itself causes oxidative injury or whether it merely makes renal epithelial cells more sensitive to agents that produce oxidative stress. Fukino et al. (1984) found that thiobarbiturate reactants, which indicate occurrence of lipid peroxidation, were markedly increased in renal cortical homogenates from rats 12 h after a s.c. injection of a nephrotoxic (15 μmol/kg) dose of mercuric chloride. Gstraunthaler et al. (1983) observed increases in the formation of malondialdehyde in renal cortical homogenates obtained from

mercuric chloride-treated rats (relative to homogenates generated from control rats treated with only cumene hydroperoxide). Because the two groups of rats were administered similar doses of mercuric chloride, concentration dependence cannot be invoked to explain the difference in observed responses. Based on these findings, it appears that inorganic mercury can enhance the ability of other agents to induced lipid peroxidation.

There are close relationships among maintenance of normal renal function, renal cellular content of glutathione, cellular redox status, and the generation of ATP in mitochondria. These relationships served as an impetus for Lund et al. (1991) to investigate the role of mercury-induced oxidative stress in mitochondria of renal epithelial cells as a mechanism for mercury-induced renal cellular injury. More specifically, they investigated the effects of inorganic mercury on the production of hydrogen peroxide by renal cortical mitochondria isolated from rats. Depending on the supply and coupling site specificity of respiratory substrates, variable increases in the formation of hydrogen peroxide were observed; incubation of isolated mitochondria with 30 nmol mercuric chloride/mg protein increased the formation of hydrogen peroxide by 4-fold at the ubiquinone-cytochrome *b* region and 2-fold at the NADH dehydrogenase region. In addition, iron-dependent lipid peroxidation was increased 3.5-fold at the NADH dehydrogenase region and by 25% at the ubiquinone-cytochrome *b* region. Intramitochondrial glutathione was decreased in a time- and concentration-dependent manner by mercuric chloride. In fact, at a concentration of 12 nmol mercury/mg protein, the content of glutathione in mitochondria was depleted completely within 30 min, suggesting that targeting of mitochondrial glutathione by mercury may be responsible for the intramitochondrial oxidative stress. Lund et al. (1993) also demonstrated that production of hydrogen peroxide, depletion of glutathione, and lipid peroxidation increased in mitochondria (isolated from renal cortical homogenates of rats treated in vivo with mercuric chloride) after the addition of an appropriate respiratory substrate. These findings support in vitro data and lead one to suggest that mercury-induced oxidative stress within mitochondria is an important mechanism involved in renal tubular injury induced by mercury.

C. Effects of Mercury on Renal Mitochondrial Function

As described earlier, Lund et al. (1991, 1993) demonstrated that inorganic mercury interferes with mitochondrial respiratory function, causing increased production of hydrogen peroxide in the mitochondria, particularly at coupling site II of the electron transport chain. Their findings indicate that an oxidative stress localized in the mitochondria may be responsible for mercury-induced inhibition of various energy-dependent processes in renal epithelial cells.

In an earlier series of studies, Weinberg et al. (1982a,b) compared the effects of mercuric chloride on mitochondrial function in vitro after either in vivo or in vitro treatment with mercuric chloride. When mitochondria were isolated from male Sprague-Dawley rats and then treated in vitro with inorganic mercury (Weinberg et al., 1982a), a marked uncoupling of respiration (i.e., increase in state 4 rate of oxygen consumption) and a significant decrease in the rate of substrate-stimulated respiration (i.e., state 3 respiration) were observed. In addition, uptake of atractyloside-insensitive ADP and the activities of both basal- and Mg^{2+}-activated oligomycin-sensitive ATPase were markedly increased by inorganic mercury. These in vitro effects occurred with a threshold concentration of mercuric chloride of 2 nmol/mg protein. Similarly, when renal cortical mitochondria were isolated from rats treated in vivo with mercuric chloride (5 mg/kg s.c.), the most prominent effects detected were inhibition of ADP uptake and decreases in the rates of state 3 and uncoupler-stimulated respiration (Weinberg et al., 1982b). These effects were not attributed to interaction of mercury with mitochondria during the isolation procedure. However, with both in vivo and in vitro treatment, inorganic mercury was not readily washed out of mitochondria, suggesting binding between mercuric ions and thiol-containing molecules in the mitochondria.

Chavez and Holguin (1988) and Chavez et al. (1991) also reported uncoupling of mitochondrial respiration after either in vivo or in vitro treatment of male Wistar rats with mercuric chloride. Consistent with this finding, they found that inorganic mercury induced calcium efflux from mitochondria, oxidation of pyridine nucleotides, and a collapse of the membrane potential. Chavez and Holguin (1988) found that inorganic mercury bonded to mitochondrial protein in a concentration-dependent manner, with saturation at approximately 9 nmol Hg^{2+}/mg protein. The finding supports the notion that a mechanism by which mercury induces mitochondrial injury is the formation of complexes between mercuric ions and mitochondrial sulfhydryl groups.

Jung et al. (1989) used ATP depletion by different chemical agents in microdissected nephron segments to localize the nephron site specificity of injury. They found that 1 μM mercuric chloride produced a significant depletion of intracellular ATP exclusively in S2 segments; nephron segments derived from the other regions of the proximal tubule (i.e., S1 or S3) or distal nephron (e.g., the distal convoluted tubule or the medullary thick ascending limb of the loop of Henle) were not as sensitive to ATP depletion after incubation with inorganic mercury. This pattern agrees with histopathological data, which demonstrate that the pars recta of the proximal tubule is the primary target of inorganic mercury, although the S3 segment is also part of the pars recta and becomes intoxicated by mercury in vivo. These data tend to support the conclusion that renal mitochondria are

early intracellular targets of inorganic mercury. This is logical considering the extremely high content of sulfhydryl-containing proteins in both the mitochondrial matrix and the inner mitochondrial membrane.

Zalups et al. (1993b) studied the accumulation and toxicity of inorganic mercury and the effects of inorganic mercury on mitochondrial function in suspensions of isolated segments of renal proximal tubules from the rabbit. Incubation of proximal tubular segments with mercuric chloride, in the absence of extracellular thiols, caused a marked time- and concentration-dependent inhibition of nystatin-stimulated oxygen consumption, demonstrating mitochondrial toxicity in an intact in vitro renal cellular model. Furthermore, inhibition of oxygen consumption by mercuric chloride preceded the development of irreversible cellular injury, as assessed by the release of lactate dehydrogenase (LDH) from the tubular segments, suggesting that inhibition of cellular energetics is a critical component of the nephrotoxic response to inorganic mercury.

D. Effects of Mercury on Intracellular Distribution of Calcium Ions

Inorganic mercury also induces the efflux of calcium ions from renal mitochondria of rats both in vivo and in vitro (Chavez and Holguin, 1988; Chavez et al., 1991). The importance of maintaining appropriate intracellular concentrations of calcium for proper cellular function is well documented, suggesting that the prominent effects of mercury on mitochondrial calcium status may play an important part in the acute nephropathy induced by mercury.

Smith et al. (1987) used primary cultures of renal tubular cells from rabbits that were mostly of proximal tubular origin as an in vitro model system to study the effects of inorganic mercury on the intracellular distribution of ionic calcium. They used the fluorescent dye Fura 2 to quantify the cytosolic content of free ionized calcium. Treatment of cells with low concentrations (2.5–10 μM) of inorganic mercury produced 2- to 10-fold increases in the intracellular content of calcium. In contrast, exposure of cells to higher concentrations (25–100 μM) of inorganic mercury produced an initial, rapid, 10- to 12-fold increase in intracellular calcium, and then the levels of calcium returned quickly to about twice those in control cells. This was followed subsequently by a second, more gradual increase in the intracellular content of calcium that was dependent on the presence of extracellular calcium. Cytotoxicity was also associated with this phase of increase in intracellular calcium and was similarly dependent on the presence of extracellular calcium. The increases in cytosolic content of calcium that were independent of extracellular concentrations of calcium were due primarily to release of intracellular calcium ions from nonmitochondrial intracellular stores, presumably derived from the endoplasmic reticulum. The subsequent decrease in intracellular calcium may be due to buffering processes, such as uptake, through the microsomal Ca^{2+},Mg^{2+}-ATPase or through the mitochondrial uniporter. The dependence of the slow, late-phase increase in cytosolic calcium on extracellular calcium associated with higher concentrations of inorganic mercury suggests that nonlethal effects of inorganic mercury in renal cells are associated with redistribution of intracellular stores of calcium. However, the toxic effects of inorganic mercury are associated with changes in permeability of the plasma membrane.

E. Alterations in Plasma Membrane (Na^++K^+)-Stimulated ATPase Induced by Mercury

Cellular plasma membranes contain a large number of proteins possessing sulfhydryl groups that are critical for enzymatic activity and membrane structure (Rothstein, 1970). Among these is the (Na^++K^+)-stimulated ATPase located on the basolateral membrane of epithelial cells in both the proximal and distal regions of the nephron, which is inhibited markedly by alkylation or oxidation of its sulfhydryl group. Anner and colleagues (Anner and Moosmayer, 1982; Anner et al., 1992; Imesch et al., 1992) conducted a detailed series of studies on the interaction between mercury-containing compounds and purified and reconstituted ATPase protein from the renal outer medulla of the rat, rabbit, and sheep. To determine the molecular details of the interaction between mercury-containing compounds and the (Na^++K^+)-stimulated ATPase, studies had to be performed with purified and reconstituted enzyme rather than intact renal epithelial cells or renal tubules.

Anner et al. (1992) showed that a number of mercury-containing compounds, including mercuric chloride, mersalyl, and *p*-mercuribenzene-sulfonic acid, potently inhibited the activity of the ATPase by binding to a site distinct from that at which the cardiac glycosides (e.g., digoxin and ouabain) bind. The binding of inorganic mercury was concentration-dependent and was modulated by the addition of chelators of heavy metal ions, such as EDTA or DMPS, indicating that the binding of inorganic mercury to the enzyme is reversible.

Imesch et al. (1992) showed that inactivation of the (Na^++K^+)-stimulated ATPase by mercuric chloride (0.1–100 μM) apparently loosens the interaction between the α- and β-subunits of the ATPase molecule, thereby altering the sensitivity of the enzyme to extracellular drugs, hormones, and antibodies.

Moreover, Anner and Moosmayer (1992) showed that the binding of inorganic mercury to the (Na^++K^+)-stimulated ATPase molecule occurs primarily at the cytosolic surface. Binding of mercury was closely correlated with inhibition of uptake of ^{86}Rb, indicating that the metal-binding site is critical to the active transport function of the ATPase.

An important extension of these studies will be to design experiments to investigate the effects of mercury on (Na^++K^+)-stimulated ATPase function in more in-

tact renal systems, such as isolated perfused tubular segments or isolated cells. Considering the potency of the interaction and the fact that the plasma membrane is a very early target site for mercury, it is likely that this interaction will be important in the mechanism of mercury-induced renal cellular injury. It is also likely that sulfhydryl groups on other membrane proteins, particularly those in the epithelial cells lining the proximal tubule, interact with mercury and may play a role in the nephropathy induced by mercury.

F. Molecular Interactions between Mercuric Ions and Aquaporins (Water Channels)

Of the aquaporins found in mammals, all except AQP4 have been shown to be sensitive to the actions of mercury (Verkman, 1992). AQP1 is present in the proximal tubule, thin descending limb of the loop of Henle and vasa recta; AQP2 (which is the vasopressin-regulated water channel), AQP3, and AQP4 are found in the collecting duct; AQP6 is found in the papilla; and AQP7 is found in the proximal tubule (Verkman, 1999). The binding of mercuric ions to these aquaporins results in the blockade of their function. This blockade is likely one of the mechanisms by which mercuric compounds, including mercurial diuretics, induce polyurea or diuresis. It had been hypothesized by Levy et al. (1958) that mercurial diuretics functioned by having the mercuric ion in the diuretic molecule bond to critical sulfhydryl and other nucleophilic groups on the tubular epithelial cells after the cleavage of the mercury-carbon bond in the diuretic molecule. A likely target is one of the cysteinyl residues in one or more of the different types of aquaporin molecules.

It is currently thought that the blockade of water channel function is indeed due to a critical change in the conformation of the protein, which results after the binding of mercury to the sulfhydryl group of one or more cysteinyl residues in that protein. Recent data collected using site-directed mutagenesis in *Xenopus* oocytes provide evidence that cysteine-11 is the mercury-sensitive residue in AQP3 (Kuwahara et al., 1997), which is found in the basolateral membrane of the collecting duct, and is involved in the transport of water and small molecules like urea. The effects of mercury on water channel function has been shown to be reversible using chelators such as 2-mercaptoethanol (Verkman, 1992). It is currently unclear, however, whether the molecular interactions that occur between mercuric ions and aquaporins play a mechanistic role in the nephropathy induced by mercury-containing compounds.

G. Influence of Mercury on Heme Metabolism

Exposure to mercury in vivo has also been shown to induce porphyrinuria (Woods et al., 1990a). The porphyrinogenic properties of mercury-containing compounds were initially attributed to metal-induced alterations in the regulation of enzymes involved in heme biosynthesis or degradation in target cells. However, because the magnitude of porphyrin excretion during prolonged exposure to either methylmercury or inorganic mercury is greater than can be accounted for by changes in heme metabolism alone, Woods et al. (1990a,b) invoked alternative biochemical mechanisms to explain their findings. They showed that mercuric ions promoted free radical-mediated oxidation of reduced porphyrins. The mechanism involved the depletion or interference of normal antioxidants in renal epithelial cells, such as endogenous thiols like glutathione. Furthermore, the ability of inorganic mercury and glutathione to react with endogenously produced reactive oxygen metabolites, from both hepatic and renal mitochondria of rats, was correlated with porphyrinogen oxidation.

An important clinical application of this effect of mercury is illustrated in a study by Bowers et al. (1992), who evaluated patterns of urinary excretion of porphyrin in male Fischer 344 rats as a diagnostic tool to assess exposure to inorganic mercury or methyl mercury. Evaluation of the urinary excretion of porphyrins is a noninvasive method that can be applied to human populations suspected of being exposed to mercury-containing compounds (Woods et al., 1993).

H. Expression of Stress Proteins after Exposure to Mercury

Various environmental stimuli, including toxic chemicals, increase the synthesis of a class of proteins known as stress proteins. Goering et al. (1992) evaluated the effect of a nephrotoxic dose of mercuric chloride (1 mg/kg) on patterns of protein synthesis in the kidneys of male Sprague-Dawley rats. Enhanced de novo synthesis of 70- and 90-kDa molecular mass proteins were detected as early as 2 h after exposure to inorganic mercury, and maximal increases in protein levels were observed at 4 to 8 h post-treatment. By 16 h postinjection, rates of synthesis of the stress proteins decreased back toward basal levels. Changes in protein expression also occurred in liver but were of smaller magnitudes and were not observed until 16 to 24 h postinjection.

Goering et al. (1992) concluded that alterations in expression of stress proteins precede overt renal injury and are target organ-specific, suggesting that they may serve as biomarkers of renal injury. Furthermore, once the biological functions of these proteins are identified, a more complete understanding of the early effects of mercury can be obtained.

More recently, Hernandez-Pando et al. (1995) provided data on the localization of 65- and 70-kDa heat shock proteins in the kidneys of rats in which acute tubular necrosis had been induced by mercuric chloride. In control rats, they found that the 65-kDa heat shock proteins were present in the cytoplasm of podocytes and proximal convoluted tubules, and the 70-kDa heat shock proteins were found in the cytoplasm and nuclei of podocytes, cortical convoluted tubules, and collecting ducts.

They demonstrated, using immunoelectron microscopy, increased expression of the 65-kDa protein in mitochondria, nuclear chromatin, and nucleoli and an overexpression of 70-kDa heat shock proteins in the cytoplasm, mitochondria, lysosomes, cytoskeleton, nuclear chromatin, and nucleoli in cortical tubular epithelial cells. During the postregenerative phase, the level of expression of the 65- and 70-kDa heat shock proteins was similar to that found in control animals. These findings indicate the induction of 65- and 70-kDa heat shock proteins are a significant component of the nephropathy induced by inorganic mercury.

I. Interactions Between Mercury and Cytoskeleton

Very little is known about the interactions between inorganic or organic mercuric ions and the cytoskeleton in renal epithelial cells. However, there are data from nonrenal cells indicating that mercury can have a significant effect on the cytoskeleton. Miura et al. (1984) demonstrated that inorganic mercury and methylmercury inhibit in vitro polymerization of tubulin. They have also demonstrated, in mouse glioma cells, that methylmercury disrupts the microtubular network at an early stage of growth inhibition. Sager and Syversen (1984) also demonstrated that disruption of microtubules occurs in the neuroblastoma, glioma, and fibroblast cell lines when they are exposed to methylmercury. Neuroblastoma cells appear to be particularly sensitive to the microtubular disruption induced by methylmercury. Microtubular damage has also been reported in lymphocytes exposed to methylmercury (Brown et al., 1988). It was suggested by Vogel et al. (1985) that methylmercury inhibits microtubular assembly by binding to free sulfhydryl groups on the ends and surface of the microtubules. The addition of the chelator DMSA appears to promote reassembly of microtubules in cells exposed to methylmercury (Sager and Syversen, 1984), presumably be removing mercuric ions from critical sulfhydryl groups.

The potential for the various forms of mercury mediating some form of toxic effect in renal epithelial cells via interactions with cytoskeletal elements remains a possibility. Inasmuch as there are numerous homeostatic functions, in addition to providing structural integrity to cells, that are carried out by various cytoskeletal components, one must consider the potential effects of mercury on the cytoskeleton when evaluating the mechanisms involved in the nephropathy induced by mercury.

V. Renal Toxicity of Mercury

All forms of mercury are nephrotoxic (Cuppage and Tate, 1967; Gritzka and Trump, 1968; Fowler, 1972; Klein et al., 1973; Ganote et al., 1974; McDowell et al., 1976; Zalme et al., 1976; Magos and Clarkson, 1977; Zalups and Diamond, 1987a; Zalups et al., 1988, 1991b; Zalups and Lash, 1990, 1994; Zalups, 1991b; Zalups and Barfuss, 1996b), although the inorganic forms of mercury are far more acutely nephrotoxic. With organic mercuric compounds, multiple exposures to relatively large doses are generally required to induce renal injury (Chang et al., 1973; Magos et al., 1985; McNeil et al., 1988). Renal injury induced by inorganic mercury is generally expressed fully during the initial 24 h after exposure and can be induced in rats with a single dose as low as 1.5 μmol Hg/kg (Zalups and Diamond, 1987b; Zalups et al., 1988). It should be pointed out, however, that rats tend to be more vulnerable to the nephrotoxic effects of inorganic mercury than New Zealand White rabbits or several strains of mice (R. K. Zalups, unpublished observations). Some strain differences in the severity of the nephropathy induced by inorganic mercury in rats also appear to exist (R. K. Zalups, unpublished observations).

In rats, the oral LD_{50} for inorganic mercury, in the form of mercuric chloride, has been reported to be in the range of 25.9 to 77.7 mg/kg (Kostial et al., 1978). A lower range of doses of inorganic mercury (10–42 mg/kg), in the form of mercuric chloride, has been estimated to be fatal in humans (Gleason et al., 1957). In one study of human poisoning with mercuric chloride, nine patients died after ingesting a single dose of inorganic mercury ranging from 29 to more than 50 mg/kg (Troen et al., 1951; World Health Organization, 1991). It should be pointed out that death due to the ingestion of a single dose of inorganic mercury is generally due to multiple effects. In addition to acute renal failure, cardiovascular collapse, shock, and severe gastrointestinal damage and bleeding are contributing causes of death.

A. Site of Tubular Injury Induced by Mercury

It is well established that the pars recta (straight segment) of the proximal tubule (particularly the portion at the junction of the cortex and outer medulla) is the segment of the nephron that is most vulnerable to the toxic effects of both inorganic and organic forms of mercury (Rodin and Crowson, 1962; Cuppage and Tate, 1967; Gritzka and Trump, 1968; Verity and Brown, 1970; Cuppage et al., 1972; Fowler, 1972; Klein et al., 1973; Ganote et al., 1974; McDowell et al., 1976; Zalme et al., 1976; Zalups and Diamond 1987a,b; Zalups et al., 1988, 1991b; Zalups and Lash, 1990; Zalups, 1991b; Zalups and Barfuss, 1996b). Depending on the severity of the nephropathy induced by mercury, cellular injury and necrosis can occur along the entire length of the pars recta, from just underneath the capsule to the junction of the outer and inner stripes of the outer medulla.

The toxic effects of inorganic forms of mercury are elicited very rapidly in the kidneys. Degenerative changes have been detected along portions of the proximal tubule of rats as early as 1 h after exposure to a very high (100 mg $HgCl_2$/kg) dose of mercuric chloride (Rodin and Crowson, 1962). At lower doses of inorganic mercury (1–5 mg $HgCl_2$/kg), significant pathological changes are

generally not detected with light microscopy until about 6 to 8 h after exposure (Rodin and Crowson, 1962; Ganote et al., 1975). At the electron microscopic level, however, cellular pathology in proximal straight tubules has been observed in rats in as little as 3 h after s.c. treatment with a 4-mg/kg dose of mercuric chloride (Gritzka and Trump, 1968). Some of the pathological features detected include mitochondrial matrix swelling with loss of matrix granules, dilation of cisternae of rough endoplasmic reticulum, loss of ribosomes from the rough endoplasmic reticulum, dispersion of ribosomes, increase in number and size of the cisternae of the smooth endoplasmic reticulum, and single membrane-limited inclusion bodies. By the end of the initial 12 h after exposure to nephrotoxic doses of inorganic mercury, cellular necrosis along the pars recta of the proximal tubule is prominent at both light and electron microscopic levels (Rodin and Crowley, 1962; Gritzka and Trump, 1968).

Convoluted portions of proximal tubules and sometimes distal segments of the nephron can be involved when the nephropathy is very severe (Rodin and Crowson, 1962; Gritzka and Trump, 1968). The involvement of segments of the nephron distal to the proximal tubule may represent secondary effects elicited by the severe damage to the pars recta of proximal tubules. However, until there are more definitive data on the direct, in vivo, toxic effects of mercuric compounds on segments of the nephron distal to the proximal tubule, the cause of distal injury in the nephron remains speculative.

If the exposure to a nephrotoxic dose of inorganic mercury is not fatal, the proximal tubular epithelium usually regenerates completely during the initial 2 weeks after the induction of tubular pathology. For example, complete relining of the proximal tubular epithelium has been demonstrated in rats as early as 4 days after receiving a 1.5 mg/kg i.v. dose of mercuric chloride (Cuppage et al., 1972).

It is interesting that in contrast to the effects of mercury in vivo, all three segments (S1, S2, and S3) of the proximal tubule (of the rabbit) become intoxicated with either inorganic mercury or methylmercury when the mercury-containing compounds are perfused through the lumen of these segments in vitro (Barfuss et al., 1990; Zalups et al., 1991a; Zalups and Barfuss, 1993a). The differences between the in vivo and in vitro findings are somewhat perplexing because all segments of the proximal tubule accumulate mercury under both experimental conditions. Another interesting difference between the in vivo and in vitro situation is that in vitro, organic mercury (specifically methylmercury) is more toxic to proximal tubular epithelial cells than inorganic mercury. This has been demonstrated in primary cultures of proximal tubular epithelial cells (Aleo et al., 1987; 1992) and in isolated perfused segments of the proximal tubule (Zalups and Barfuss, 1993a).

B. Markers of Renal Cellular Injury and Impaired Renal Function Induced by Mercury

A number of methods have been used to detect renal tubular injury induced by mercury. One noninvasive method that has been used frequently is to measure the urinary excretion of a number of cellular enzymes (Ellis et al., 1973; Planas-Bohne, 1977; Stroo and Hook, 1977; Kirschbaum, 1979; Buchet et al., 1980; Price, 1982; Stonard et al., 1983; Gottelli et al., 1985; Zalups and Diamond, 1987b). The rationale for using the urinary excretion of cellular enzymes as an indicator of renal tubular injury is based on the close association between renal cellular necrosis and enzymuria. After renal epithelial cells have undergone cellular necrosis, most, if not all, of the contents of the necrotic epithelial cells, including numerous cellular enzymes, are released into the tubular lumen and are excreted in the urine. The usefulness of any particular cellular enzyme as a marker of renal cellular injury or necrosis depends on the stability of the enzyme in urine, whether the enzyme or the activity of the enzyme is greatly influenced by the toxicant that is being studied, and the subcellular localization of the enzyme relative to the subcellular site of injury.

During the early stages of the nephropathy induced by mercury, before tubular necrosis, cells along the proximal tubule undergo a number of degenerative changes and begin to lose some of their luminal (brush-border) membrane (Zalme et al., 1976). Evidence from several studies shows that the urinary excretion of the brush-border enzymes, alkaline phosphatase and γ-GT, increases during the nephropathy induced by mercury-containing compounds (Price, 1982; Gotelli et al., 1985; Zalups et al., 1988, 1991b). When tubular injury becomes severe and necrosis of tubular epithelial cells is apparent, the urinary excretion of a number of intracellular enzymes, such as LDH, aspartate aminotransferase, alanine aminotransferase, and *N*-acetyl-β-D-glucosaminidase, increases (Planas-Bohne, 1977; Zalups and Diamond, 1987b; Zalups et al., 1988, 1991b; World Health Organization, 1991; Agency for Toxic Substance and Disease Registry, 1994).

After a significant number of proximal tubules have become functionally compromised by the toxic effects of mercury, the capacity for the reabsorption of filtered plasma solutes and water is greatly diminished. As a consequence of this diminished absorptive capacity, there is increased urinary excretion of both water and a number of plasma solutes, such as glucose, amino acids, albumin, and other plasma proteins (Price, 1982; Zalups and Diamond, 1987b; Diamond, 1988; Zalups et al., 1988). In a recent study of workers exposed to mercury vapor, it was demonstrated that increased urinary excretion of Tamm-Horsfall glycoprotein and tubular antigens and decreased urinary excretion of prostaglandins E_2 and $F_{2\alpha}$ and thromboxane B_2 can also be used as

indices of renal pathology induced by mercury (Cardenas et al., 1993).

In two reports, the urinary excretion of mercury (factored by the total renal mass) was demonstrated (in normal and uninephrectomized rats) to correlate very closely with the level of injury in pars recta segments of proximal tubules during the acute nephropathy induced by low toxic doses of inorganic mercury (Zalups and Diamond, 1987b; Zalups et al., 1988). In these reports, the urinary excretion of mercury was shown to correlate with the histopathological scoring of injury to the pars recta of proximal tubules and increased urinary excretion of albumin, total protein, and the cellular enzymes LDH, γ-GT, and *N*-acetyl-β-D-glucosaminidase (Zalups and Diamond, 1987b; Zalups et al., 1988). Overall, it appears that as the level of renal injury increases in the kidneys, there is a corresponding increase in the urinary excretion of mercury. Other nephrotoxic agents have also been shown to decrease the retention of mercury in the kidney and to increase the excretion of mercury in the urine (Clarkson and Magos, 1967; Magos and Stoychev, 1969; Trojanowska et al., 1971), presumably by causing the release of mercury from, and decreased luminal uptake by, renal epithelial cells undergoing necrosis. Although the urinary excretion of mercury appears to correlate well with the level of acute renal injury induced by mercuric chloride, there does not appear to be a close correlation between the severity of renal injury and the renal concentration or content of mercury (Zalups and Diamond, 1987b; Zalups et al., 1988).

When renal tubular injury becomes severe during the nephropathy induced by mercury, the concentration of creatinine in plasma increases due to a decrease in glomerular filtration rate (GFR) (Barenberg et al., 1968; McDowell et al., 1976; Zalups et al., 1991b). The mechanisms responsible for the decreased GFR are not known at the present but are likely complex and involve a number of factors. In addition to causing decreases in GFR, mercury causes the fractional excretion of sodium and potassium to increase (McDowell et al., 1976). These functional changes likely reflect a significant decrease in the number of functioning nephrons, inasmuch as similar changes occur in rats and mice when their total renal mass has been reduced surgically by approximately 75% (Zalups et al., 1985; Zalups, 1989; Zalups and Henderson, 1992). As part of the severe nephropathy induced by mercury, blood urea nitrogen (BUN) also increases as plasma creatinine increases, due to the significant decreases in GFR. Thus, the measure of plasma creatinine and/or BUN may be used as an indicator of impaired renal function induced by mercury (McDowell et al., 1976). However, it is preferable to use the clearance of creatinine or inulin over the measurement of BUN as an index of renal function. BUN can be elevated by more nonrenal causes than creatinine and therefore is not as sensitive an indicator of renal function. After exposure to high doses of mercury, an oliguric or anuric acute renal failure ensues. The factors that lead to acute renal failure are complex, involving multiple systems. Clearly, further research is needed to better understand the mechanisms involved in the induction of acute renal failure induced by exposure to mercury.

C. Mercury-Induced Renal Autoimmunity

There is evidence from studies with rabbits (Roman-Franco et al., 1978), inbred Brown-Norway rats (Druet et al., 1978), and a cross between Brown-Norway and Lewis rats (Bigazzi, 1988, 1992) that multiple exposures to inorganic mercury can lead to the production of antibodies against the glomerular basement membrane and results in an immunologically mediated membranous glomerular nephritis. This glomerular nephropathy is characterized by the binding of antibodies to the glomerular basement membrane, followed by the deposition of immune complexes in the glomerulus (Sapin et al., 1977; Druet et al., 1978; Roman-Franco et al., 1978). There also is evidence from studies implementing several strains of both mice and rats that repeated exposures to inorganic mercury can lead to the deposition of immune complexes in the mesangium and glomerular basal lamina, which leads to an immune complex glomerulonephritis (Weening et al., 1981; Enestrom and Hultman, 1984; Bigazzi, 1988; Hultman and Enestrom, 1992). Whether mercury can induce an autoimmune glomerulonephritis in humans is not clear at the present. It should be pointed out that a majority of the cases of glomerulonephritis (of an immunological origin) in humans is classified as idiopathic. Thus, until research proves otherwise, it remains possible that some forms of glomerulonephritis could be induced by exposure to mercury or other environmental or occupational toxicants.

The autoimmunity induced by mercury likely reflects some complex effects of mercuric ions on cell-signaling and gene expression events in immune cells, such as in monocytes and lymphocytes. For example, Koropatnick and Zalups (1997) recently demonstrated that the exposure of human monocytes to low, nontoxic doses of the inorganic mercury causes a rapid suppression of activation signaling events that are normally induced in these cells by lipopolysaccharide or phorbol ester.

VI. Factors that Modify Renal Toxicity of Mercury

A. Influence of Intracellular Thiols on Renal Accumulation and Toxicity of Mercury

Two major intracellular thiols, glutathione and metallothionein, appear to be important in regulating the renal accumulation of mercury and, ultimately, the susceptibility to mercury-induced renal cellular injury. It is likely that other molecules within cells, including the large supply of nonmetallothionein, protein sulfhydryls,

play some role in the renal cellular accumulation and toxicity of mercury.

Intracellular concentrations of glutathione can be manipulated readily within a relatively brief period in time. Several investigators have used diethyl maleate to conjugate glutathione, thereby lowering the amount of intracellular glutathione available to interact with mercuric ions. Johnson (1982), Berndt et al. (1985), Baggett and Berndt (1986), Zalups and Lash (1997), and Zalups et al. (1999a,b,c) demonstrated that the depletion of intracellular glutathione or nonprotein thiols is accompanied by decreases in the renal accumulation of inorganic mercury in animals treated with mercuric chloride. In the studies by Berndt and colleagues (Berndt et al., 1985; Baggett and Berndt, 1986), the depletion of intracellular glutathione appeared to increase the severity of renal injury induced by treatment with mercuric chloride. Zalups and Lash (1990) also found a close correlation between intrarenal concentrations of glutathione and the accumulation of inorganic mercury. There are some conflicting findings on the effects of diethyl maleate from the laboratory of Girardi and Elias (1991), who reported increases in renal accumulation of inorganic mercury in mice treated with this compound. Recently, Zalups and Lash (1997b) and Zalups et al. (1999a,b,c) demonstrated in rats that the acute depletion of glutathione in the kidneys and liver by treatment with diethyl maleate caused significant decreases in the renal uptake and accumulation of mercury during the initial hour after the administration of low nontoxic dose of mercuric chloride. Interestingly, although the renal accumulation of mercury decreased after treatment with diethyl maleate, the net hepatic accumulation of mercury increased. Thus, the depletion of renal and hepatic glutathione has mixed effects on the disposition of mercury.

In other experiments, Tanaka-Kagawa et al. (1993) lowered the intracellular content of glutathione in the kidneys of mice by administering buthionine sulfoximine (which is a potent inhibitor of γ-GCS, which is the rate-limiting enzyme in the intracellular synthesis of glutathione), and then inhibited extracellular degradation of glutathione by γ-GT using acivicin. They observed no changes in the accumulation of either inorganic mercury or methylmercury compared with control animals. Zalups and Lash (1997b) and Zalups et al. (1999a) have also shown in rats that the acute depletion of renal glutathione with buthionine sulfoximine does not affect the early aspects of the accumulation of inorganic mercury in the kidneys. By contrast, Zalups et al. (1999b,c) demonstrated that pretreatment with buthionine sulfoximine did cause significant decreases in the net renal content of mercury 24 h after treatment with inorganic mercury (Zalups et al., 1999b,c). These findings indicate that there are significant temporal factors with respect to the effects of buthionine sulfoximine on the renal disposition of mercury.

In studies in which acivicin was used to inhibit γ-GT, Berndt et al. (1985) and Zalups (1995) showed in rats and Tanaka et al. (1990) showed in mice that the urinary excretion of glutathione and inorganic mercury increased after the inhibition of glutathione degradation. Tanaka-Kagawa et al. (1993) also found that the urinary excretion of inorganic mercury increased, whereas the renal accumulation of either inorganic mercury or methylmercury decreased.

Tanaka et al. (1990) also found that when mice were pretreated with 1,2-dichloro-4-nitrobenzene, to deplete the hepatic content of glutathione (before injection of mercuric chloride), there was a marked reduction in the renal accumulation of mercury and a significant decrease in the level of renal cellular injury induced by inorganic mercury. These findings tend to suggest that hepatically synthesized glutathione and the activity of γ-GT are involved in the renal uptake of mercury. Additional findings from a set of recent studies in which bile flow was either diverted or prevented from entering the small intestine of rats demonstrate that some aspect of hepatic function is linked to a component of the renal uptake and accumulation of mercury (Zalups and Barfuss, 1996a; Zalups, 1998a).

Increases in the intracellular contents of glutathione and other nonprotein thiols can be achieved by several means. Girardi and Elias (1991, 1993) reported that the treatment of mice with *N*-acetylcysteine caused decreased intracellular accumulation of inorganic mercury in both the kidneys and liver. Inasmuch as hepatic transport of inorganic mercury with glutathione has been established in liver, higher intracellular contents of glutathione would be expected to provide increased numbers of ligands for binding to inorganic mercury. The seemingly paradoxical results of Girardi and Elias (1991) and the discrepancies described earlier suggest that the intrarenal disposition of mercury-containing compounds must be regulated by a more complex array of factors than the availability of reduced glutathione.

Acute biliary ligation has also been shown to cause significant increases in the renal and hepatic content of glutathione in rats (Zalups et al., 1999c). Zalups et al. (1999c) suggested that the observed increased renal concentration of glutathione induced by biliary ligation was due to a hepatic mechanism. They believed that as the concentration of glutathione in the biliary canaliculi increased (after biliary ligation), the transport of glutathione out of the hepatocytes was redirected down a concentration gradient into the sinusoidal blood. They also believed that as glutathione was continually added to the blood, plasma concentrations of this thiol increased, which provided more glutathione to be taken up at the luminal and basolateral membranes of proximal tubular epithelial cells in the kidneys. Interestingly, biliary ligation was shown to cause the net accumulation of mercury in the liver to increase and the net accumulation of mercury in the kidneys to decrease during the initial

24 h after the i.v. injection of 0.5 μmol $HgCl_2$/kg. What makes these findings interesting is that the renal accumulation of mercury was decreased despite an increased renal cellular content of glutathione, which is contrary to what one might expect. It was postulated that the decreased renal accumulation of mercury in animals that had undergone biliary ligation was not due to the content of glutathione in the kidney but rather the content of glutathione in the liver, where the accumulation of mercury had increased. These findings also confirm that some aspects of hepatic function play a role in the renal disposition of mercury.

Additional experiments by Tanaka-Kagawa et al. (1993), in which intracellular levels of metallothionein were modulated, may provide some clarification of the contradictory reports on the effects of glutathione depletion on the renal accumulation of mercury. These investigators found that induction of renal metallothionein with $Bi(NO_3)_3$ diminished the ability of acivicin to decrease intrarenal accumulation of either inorganic mercury or methylmercury. They interpreted this as indicating that inorganic mercury or methylmercury that is bound to ligands other than metallothionein in renal cells can be secreted readily into the tubular lumen with intracellular glutathione. Other studies (Fukino et al., 1984, 1986; Zalups and Cherian, 1992a,b) documented that the induction of renal metallothionein is associated with increased intrarenal accumulation of mercury and decreased severity of the nephropathy induced by either organic or inorganic mercury. Thus, it appears there is a complex interplay between protein and nonprotein thiols in the renal disposition mercury.

B. Modulation of Renal Accumulation and Toxicity of Mercury by Extracellular Thiols

Although manipulation of intracellular thiols is sometimes used therapeutically to alter the accumulation of mercury and to modulate effects of mercury once it enters target sites, the administration of thiol-containing compounds can be applied before or simultaneously with mercury-containing compounds to alter the pharmacokinetics and pharmacodynamics of mercury. Both DMPS and DMSA are becoming two of the metal chelators more commonly used as antidotes for mercury poisoning, and their chemical and pharmacological properties have been reviewed by Aposhian (1983) and Aposhian and Aposhian (1990). Examples of some of their most distinguishing features are that in contrast to the earlier chelator dimercaprol (also known as British Anti-Lewisite), DMPS and DMSA are fairly nontoxic, are very water soluble, are not very lipid soluble, and are effective if administered orally. The two compounds are quite versatile, being capable of chelating arsenic, lead, cadmium, and mercury. However, they differ in potency and specificity; for example, DMPS is generally more effective of the two in chelating inorganic forms of mercury (Planas-Bohne, 1981; Zalups, 1993b). Additional extracellular thiol reagents that have been used clinically for the removal of methylmercury are D-penicillamine and *N*-acetyl-DL-penicillamine (Aposhian, 1983). Some of the reported variability in effectiveness and potency of the various chelators of mercury may be attributed to species differences, routes of administration, and doses of chelators given.

Zalups et al. (1991b) demonstrated dose-dependent protection with DMPS in rats from the nephropathy induced by inorganic mercury. Their data suggest that the protective effects of DMPS are attributed to decreases in the renal burden of mercury and increases in the urinary excretion of mercury. Furthermore, Maiorino et al. (1991) demonstrated a high correlation between the effectiveness of DMPS and urinary excretion of both inorganic mercury and DMPS in humans. In a recent study (Zalups, 1993b), the same dose of DMPS or DMSA, when administered to rats 24 h after the animals had received an i.v. nontoxic dose of mercuric chloride, was shown to reduce the renal burden of mercury significantly during the subsequent 24 h after treatment with the respective chelator. Treatment with DMPS caused a reduction in the renal burden of mercury by more than 80%, whereas DMSA caused a reduction in the renal burden of mercury by about 50%. These findings indicate that DMPS is more effective (on a per-mole basis) in reducing the renal burden of mercury when administered after an exposure to inorganic mercury. The kinetics involved in the rapid reduction of the renal burden of mercury, after treatment with DMPS or DMSA, appear to indicate that transport of both of these chelating agents by the epithelial cells along the proximal tubule is involved in the reduction in the renal tubular burden of mercury. It is well established that both organic anions, such as sulfonates, and dicarboxylic acids, such as succinic acid, are transported by proximal tubular epithelial cells.

In a recent mechanistic study using isolated perfused proximal tubular segments, Zalups et al. (1998) provide data indicating that DMPS is taken up rapidly at the basolateral membrane by the *para*-aminohippurate-dependent organic anion transport system. The findings also show that once inorganic mercury binds to DMPS, the mercuric conjugates are not taken up readily at either the luminal or basolateral membranes. These particular findings are contrary to the commonly held presumption that mercuric conjugates of DMPS might be transported by the organic anion transport system (Zalups, 1993b). Perhaps the most important findings from this study are those indicating that DMPS can extract accumulated inorganic mercury from proximal tubular cells while it is being transported in a secretory manner from the basolateral to the luminal side of proximal tubular epithelial cells. Figure 5 illustrates the mechanisms involved in the renal cellular transport of DMPS and the mechanisms by which DMPS reduces the renal tubular burden of mercury.

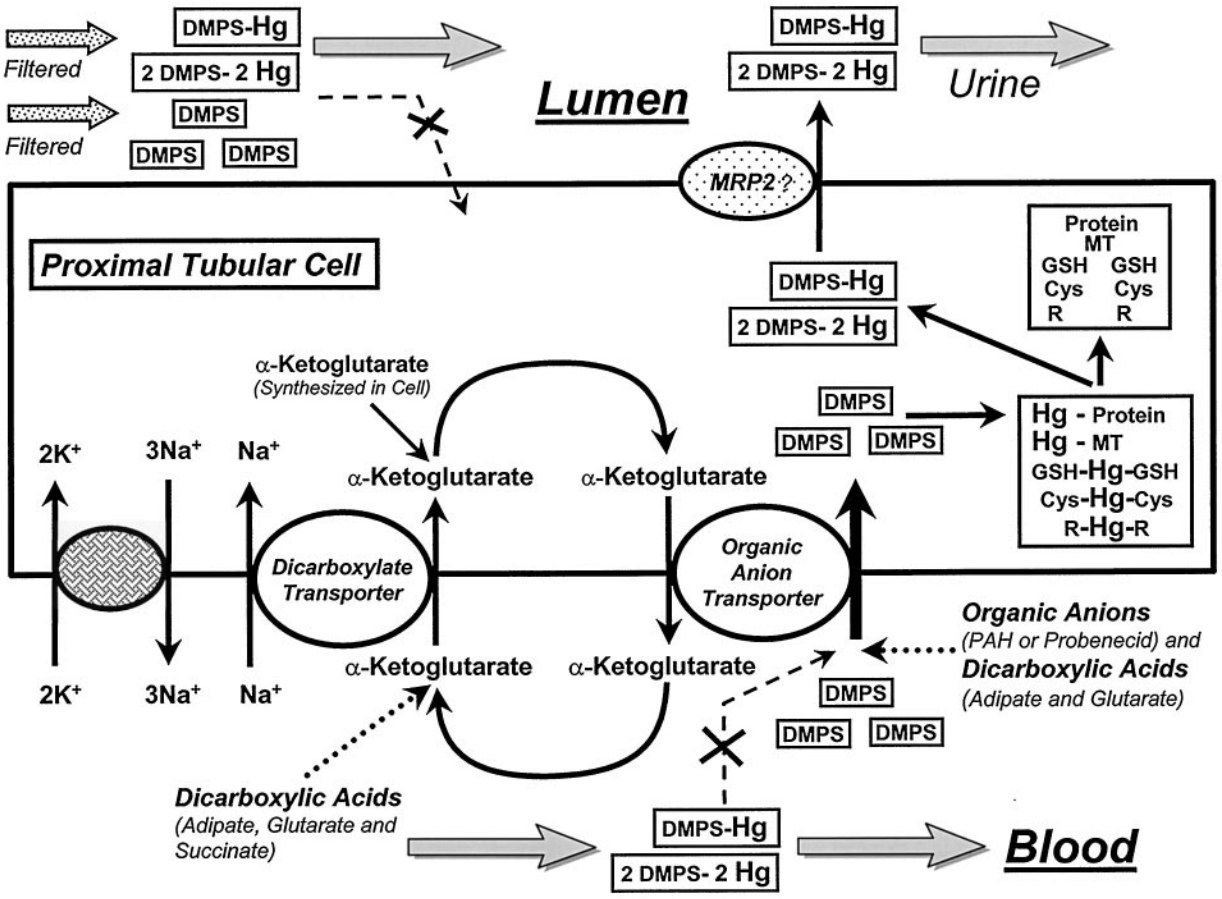

FIG. 5. DMPS is a very effective chelating agent for the removal of inorganic mercury from the kidneys. What is of interest is that the primary sight of action of DMPS is also the primary sight where both inorganic and organic forms of mercury accumulate, namely along the proximal tubule. However, the mechanisms by which DMPS reduces the renal tubular burden of mercury has been elucidated only recently. After systemic treatment with DMPS, both DMPS and mercuric conjugates of DMPS (DMPS-Hg, 2 DMPS-2 Hg, and/or other forms) are present in the blood. These compounds are filtered readily at the glomerulus and delivered to the luminal compartment of proximal tubules. They are also delivered to the basal compartment of proximal tubules via blood flow. Because of the polar negative charge associated with the sulfonate group of DMPS, it appears that neither DMPS nor mercuric conjugates of DMPS are transported readily at either the luminal or basolateral plasma membrane of proximal tubular cells. It is not surprising that mercuric conjugates of DMPS are not transported at the luminal plasma membrane because of their negative charge. However, it is surprising that mercuric conjugates of DMPS are not readily transported by the organic anion transporter, despite the fact that DMPS itself is readily transported into proximal tubular cells by this transport system (details of which are described in the legend for Fig. 4). Current evidence indicates that the therapeutic actions of DMPS involves the following steps: 1) DMPS (in a reduced and/or oxidized state) is taken up avidly at the basolateral membrane by the organic anion transporter. 2) The reduced form of DMPS interacts with, competes for, and then removes mercuric ions bonded to a host of potential molecules, which include proteins, metallothioneins (MT), glutathione (GSH), cysteine (Cys), and others (R-Hg-R). 3) Once a mercuric ion becomes bonded to DMPS and a sufficient intracellular concentration of mercuric conjugates of DMPS have formed to generate a gradient favoring the outward movement of these complexes, the conjugate is transported, likely in a facilitative manner, into the proximal tubular lumen (perhaps by MRP2). 4) Finally, the conjugates are excreted into the urine because they cannot be taken up by any segment of the nephron or collecting duct.

Additional support for the hypothesis that transport of DMPS and intracellular chelation of mercury occur along segments of the proximal tubule after treatment with DMPS comes from the study by Klotzbach and Diamond (1988). Using isolated perfused kidneys from male Long-Evans rats, they showed that DMPS undergoes net tubular secretion by a kinetically saturable process that is inhibited by *para*-aminohippurate and probenecid. They also found that DMPS produced a dose-dependent decrease in the retention of inorganic mercury and an increase in urinary excretion of inorganic mercury. Furthermore, both effects were blocked by probenecid, suggesting that the mechanism of protection by DMPS is via chelation of inorganic mercury within proximal tubular cells. Many investigators have observed that DMPS is readily oxidized in perfusates or in plasma to the disulfide form. To enable interaction with metals, DMPS is reduced back to the dithiol form within proximal tubular cells by a glutathione-dependent thiol-disulfide exchange reaction (Klotzbach and Diamond, 1988; Stewart and Diamond, 1988).

Other low-molecular-weight thiols have been used experimentally to modulate the nephrotoxicity of mercury. Because of its prominence as the primary intracellular, nonprotein thiol, exogenous glutathione is a logical choice to try as a modulatory agent. Work by Jones and colleagues (Aw et al., 1991) demonstrated that the oral administration of glutathione can significantly increase the content of glutathione in the lung, kidney, heart, brain, small intestine, and skin, but not in liver under

conditions where glutathione is depleted. This suggests that glutathione taken orally may supplement cellular glutathione in some tissues under certain toxicological or pathological conditions. A large body of data from both in vivo and in vitro systems indicate that exogenous glutathione can protect against mercury-induced renal injury. Zalups et al. (1991a) perfused isolated rabbit proximal tubules with 18.4 μM mercuric chloride and various thiols, including glutathione or cysteine. Both thiols, when present in the perfusate at a 4-fold higher concentration than inorganic mercury, either prevented or significantly decreased the extent of acute tubular injury induced by unbound mercuric ions. An ultrafiltrate of rabbit plasma was similarly protective. The mechanism of protection by glutathione, cysteine, or plasma ultrafiltrate appeared to involve decreased uptake of inorganic mercury across the luminal membrane and subsequent accumulation. Houser and Berndt (1988) administered glutathione monoethyl ester to rats and found that both renal cortical accumulation of inorganic mercury and the severity of mercury-induced renal injury were diminished.

The protective effects of exogenous glutathione and DMPS have also been demonstrated in suspensions of isolated proximal tubular cells from rats (Lash and Zalups, 1992). Proximal tubular cells were first incubated for 15 min in an extracellular buffer containing bovine serum albumin and various concentrations of glutathione or DMPS. They were then incubated for an additional 1 h in the presence of 250 μM mercuric chloride, which was found to be the threshold concentration of inorganic mercury that produced cellular injury under the incubation conditions being studied. Glutathione provided concentration-dependent protection from mercury-induced cytotoxicity, as assessed by decreases in the activity of total cellular lactate dehydrogenase. A glutathione concentration of 500 μM, or twice that of inorganic mercury, was required to completely protect proximal tubular epithelial cells. DMPS, in contrast, provided complete protection against 250 μM mercuric chloride at a concentration (175 μM) that was less than that of inorganic mercury. Differences in the level of protection afforded by glutathione and DMPS likely arise from differences in the chemistry and renal handling of the two compounds. Additional findings obtained from isolated proximal tubular epithelial cells from both normal and uninephrectomized rats have recently confirmed the protective effects of both glutathione and DMPS against the cytotoxic effects of inorganic mercury in vitro (Lash et al., 1999).

In contrast to the in vitro data described above, Tanaka et al. (1990) found that the coadministration of glutathione and mercuric chloride to mice caused the renal content of mercury to increase relative to that in mice that received mercuric chloride alone. These investigators concluded that the transport of inorganic mercury to the kidney may occur as a mercury-glutathione complex and that the simultaneous presence of glutathione enhances uptake of mercury. Zalups and Barfuss (1995b,c) observed similar effects in rats coadministered a nontoxic dose of inorganic mercury with glutathione or cysteine. Consistent with these findings, Miller and Woods (1993) showed recently that complexes of glutathione and Hg^{2+} or glutathione disulfide and Hg^{+} promoted uroporphyrinogen oxidation and catalyzed decomposition of hydrogen peroxide, indicating that mercury-glutathione (or other thiol) complexes likely contribute to mercury-induced toxicity. Some of these results have also been confirmed in rats by R. K. Zalups (unpublished observations). Zalups and Barfuss (1996b) have very recent data from work on rats indicating that when a toxic 2.0 μmol/kg dose of mercuric chloride is coadministered with cysteine, the nephropathy induced by the inorganic mercury is made more severe. Resolution of the marked contrast between these findings and in vitro findings described earlier will require a detailed mechanistic description of the renal transport of inorganic mercury. Although advances have been made in the understanding of mechanisms of renal transport of mercury, the role of thiols in the renal cellular uptake of mercury is still somewhat unclear.

In contrast to the highly effective protective effects of DMPS and DMSA against mercury-induced renal cellular injury, less definitive results have been obtained with two other dithiols, such dithioerythritol and dithiothreitol. On the one hand, Barnes et al. (1980) observed, in rats, evidence of protection against morphological lesions and losses of activities of key marker enzymes for plasma membrane and mitochondria induced by mercury with dithiothreitol. Weinberg et al. (1982a) provided evidence of protection for isolated renal mitochondria from mercuric chloride-induced dysfunction by dithioerythritol but only if the dithiol was added in vitro simultaneously with mercuric chloride; when the dithiol agent was added in vitro after the rats had been treated with mercuric chloride in vivo, no protection or reversal of toxicity was observed. To complicate further the understanding of how dithiols interact with mercury-containing compounds in biological systems, Chavez and Holguin (1988) reported that the addition of dithiothreitol to renal mitochondria isolated from the rat that had been treated with inorganic mercury actually increased the degree of mitochondrial injury induced by mercury. They suggested that the dithiol made additional sulfhydryl-sensitive sites available for interaction with mercury, thereby enhancing the toxic response. In the same study, the investigators also reported that the monothiol 2-mercaptoethanol also enhanced mercuric chloride-induced mitochondrial injury, although higher concentrations than those of the dithiol were required to reproduce the effect.

Chavez et al. (1991) also reported that the angiotensin-converting enzyme inhibitor captopril [1-(3-mercapto-2-methyl-1-oxopropyl)-1-proline] was an effective

protective agent both in vivo and in vitro against mercuric chloride-induced mitochondrial injury and morphological damage.

Although the administration of inorganic mercury complexed to the small sulfhydryl-containing protein metallothionein has not been shown to provide protection against the toxicity induced by inorganic mercury, it has been shown to alter the renal site of injury (Chan et al., 1992). The primary target of renal injury induced by mercuric chloride is the pars recta (S2 and S3 segments) of the proximal tubule, but the primary target of renal injury induced by mercury-metallothionein appears to be the pars convoluta and early pars recta (S1 and S2 segments) of the proximal tubule. Intrarenal accumulation and urinary excretion of inorganic mercury in rats has also been demonstrated to be greater when mercury was administered with metallothionein than when mercury was administered alone (Zalups et al., 1993a).

C. Effects of Reduced Nephron Number and Compensatory Tubular Hypertrophy on Renal Disposition and Toxicity of Mercury

Reduction in the number of functioning nephrons, which can occur as a consequence of aging, renal disease, or surgical removal of renal tissue, has profound effects on renal cellular function and consequently, on the renal handling of exogenous chemicals, and on the susceptibility of renal tissue to chemically induced injury (Meyer et al., 1991). After a significant loss of renal mass, the remnant renal tissue undergoes compensatory growth, which is due predominantly (i.e., >85%) to cellular hypertrophy (rather than cellular hyperplasia), particularly in segments of the proximal tubule. One of the more prominent changes in renal function that occur as a result of compensatory renal growth includes marked increases in mitochondrial metabolism, which may lead to an enhanced susceptibility of renal tissue to oxidative stress (Nath et al., 1990).

Numerous animal studies have shown that rats that have undergone a significant reduction in renal mass, such as unilateral nephrectomy, are more susceptible to the nephropathy induced by inorganic mercury than are rats with two normal kidneys (Houser and Berndt, 1986, 1988; Zalups et al., 1988; Zalups and Lash, 1990; Lash and Zalups, 1992, 1994). The biochemical changes that occur as a consequence of reduced renal mass and compensatory renal growth are retained in vitro when proximal tubular cells are isolated from rats (Lash and Zalups, 1992, 1993). Furthermore, the enhanced susceptibility of hypertrophied proximal tubular cells to the toxic effects of inorganic mercury is also retained in vitro. In the absence of exogenous thiols in the extracellular incubation medium, proximal tubular cells isolated from unilaterally nephrectomized (NPX) rats, in which compensatory renal growth had occurred, exhibited irreversible cellular injury at significantly lower concentrations of mercuric chloride than proximal tubular cells isolated from sham-operated rats.

Although the mechanism or mechanisms for the enhanced susceptibility of proximal tubular cells from NPX rats to injury induced by mercury are not well characterized, it appears that enhanced accumulation of mercury is a contributing factor. Findings from studies with both mercuric chloride (Zalups and Diamond, 1987b; Zalups et al., 1988; Zalups and Lash, 1990; Zalups, 1991c) and methylmercuric chloride (Zalups et al., 1992) indicate that greater amounts of mercury, on a per-gram tissue basis, accumulate in the remnant kidney of NPX rats than in the kidneys of sham-operated or control rats. Moreover, the findings indicate that greatest increase in the accumulation of mercury occurs in the outer stripe of the outer medulla, specifically in pars recta segments of proximal tubules (Zalups, 1991b), which coincides with the site at which the toxicity of mercury is expressed in the kidney. Other factors, such as changes in intrarenal handling of mercury, are also probably involved in changing the cellular response to mercury exposure. Some of the altered accumulation of mercury that occurs in the remnant kidney is probably related to alteration in the renal concentrations of intracellular thiols. Recent findings show that the intracellular metabolism of both glutathione (Zalups and Veltman, 1988; Zalups and Lash, 1990) and metallothionein (Zalups and Cherian, 1992a,b; Zalups et al., 1995) are altered significantly after renal mass is reduced after unilateral nephrectomy and compensatory renal growth. Zalups and Lash (1990) have shown that the cellular content of glutathione in the remnant kidney increases after uninephrectomy, especially in the outer stripe of the outer medulla. This increase in renal cellular glutathione has been shown recently to be linked to increased activity of γ-GCS (Lash and Zalups, 1994), which is the rate-limiting enzyme involved in the intracellular synthesis of glutathione. With respect to metallothionein, recent molecular biological data indicate clearly that the increased renal cellular contents of metallothionein that occur after uninephrectomy are linked directly to increased transcription of the genes for metallothionein-1 and -2 (Zalups et al., 1995).

Despite the significant progress that has been made in defining the biochemical and physiological changes that occur during compensatory renal growth, much more research is needed to understand the precise mechanisms responsible for the increased proximal tubular uptake of, and susceptibility of renal injury to, inorganic mercury that occur when renal mass has been reduced significantly.

VII. Summary

Toxicology of heavy metals encompasses a large field of research and is of interest to many because of the widespread environmental distribution of these toxi-

cants. This is particularly true of mercury-containing compounds. Significant advances have been made during the past decade, in part due to the development and validation of various in vitro biological preparations, including isolated and perfused microdissected segments of the nephron and isolated cellular suspensions and culture techniques. Principal areas that were discussed include renal accumulation and transport of mercury, molecular interaction of mercury in renal epithelial cells, renal excretion and toxicity of mercury, and factors that influence the renal toxicity of mercury.

A. Renal Accumulation and Transport of Mercury

In summary, the kidneys are one of the primary sites for the accumulation of various forms of mercury. Inorganic and organic forms of mercury accumulate primarily in the renal cortex and outer stripe of the outer medulla. Most of the accumulation of mercury in the cortex and outer stripe of the outer medulla occurs mainly along the three segments (S1, S2, and S3) of the proximal tubule. At present, there appears to be at least two primary mechanisms involved in the uptake of mercuric ions by proximal tubular epithelial cells. One of the mechanisms is localized on the luminal plasma membrane and involves the activity of the γ-GT. Mercuric conjugates of cysteine, in particular dicysteinylmercury, appear to be the primary species of mercury that are taken up most avidly at the luminal plasma membrane. It appears that at least two amino acids transport systems are involved in the luminal uptake of mercuric conjugates of cysteine: one is a sodium-dependent transport system, and the other is a sodium-independent system. At least some component of the luminal uptake of dicysteinylmercury appears to occur through the transporter or transporters involved in the luminal absorption of cystine, via a mechanism involving molecular homology. At the basolateral membrane, uptake of mercury involves the dicarboxylate and organic anion transport systems. Likely species of mercury that are transported at the basolateral membrane include mercuric conjugates of glutathione, cysteine, homocysteine, and *N*-acetylcysteine. It is not known at the present whether other segments of the nephron and collecting duct play a significant role in the renal uptake, transport, accumulation, and excretion of mercury.

To understand the mechanisms involved in the tubular uptake of mercury, one must consider and understand the molecular interactions that occur between the various forms of mercury and sulfhydryl-containing molecules that are present in various compartments in the body. To further emphasize this point, one only needs to view the current body of evidence on the renal tubular transport of mercury, which shows overwhelmingly that mercury is likely cotransported into renal tubular (proximal) epithelial cells with thiol-containing compounds. However, further work is needed to establish the relationship between the renal cellular uptake of mercury and the interactions and relationships between mercurous and mercuric ions and cysteine, glutathione, metallothioneins, albumin, and other ligands (containing sulfhydryl groups) in the different compartments of the body.

B. Molecular Interactions with Mercury in Renal Epithelial Cells

Although many of the biological effects of mercury-containing compounds in renal tissues can be attributed to the binding of mercury to plasma membrane or intracellular thiols, many findings are inconsistent with this being the sole mechanism of action. It has been established that through alterations in intracellular thiol metabolism, mercury can promote oxidative stress, lipid peroxidation, mitochondrial dysfunction, and changes in heme metabolism.

Conner and Fowler (1993) attempted to explain some of the biological effects of mercury-containing compounds in renal tissue. In their scheme, after mercuric or methylmercuric ions enter proximal tubular epithelial cells via transport across either the brush-border or basolateral membrane or membranes, or both, they interact with thiol-containing compounds, principally glutathione and metallothionein. They proposed that the early effects of mercury include alterations in membrane permeability to calcium ions and inhibition of mitochondrial function. Moreover, they propose that through unknown signaling mechanisms, mercury induces the synthesis of glutathione, various glutathione-dependent enzymes, metallothionein, and several stress proteins (in kidneys and liver). It is important to realize that the inductive effects of mercuric or methylmercuric ions occur primarily at nontoxic to moderately toxic doses of mercury; at higher doses, cellular injury occurs and biosynthetic processes are inhibited. Although this model accounts for some of the data in the literature, little information regarding the mechanisms of transport of mercury across luminal and basolateral membranes of renal tubular epithelial cells or certain biochemical effects of mercury in renal tubular epithelial cells, such as oxidative stress, is provided. Hence, although this model explains some effects of mercury and illustrates how some of these processes may interact to produce renal cellular injury, a more thorough integrated model is needed to explain the biochemical mechanisms involved in mercury-induced renal cellular injury.

C. Renal Toxicity of Mercury

Despite compelling histopathological data implicating the pars recta of the proximal tubule as the primary target site that is adversely affected by mercury, other segments of the nephron may also be involved in the nephropathy induced by mercurials. To date, however, little work has been performed on in vitro systems derived from nephron segments other than the proximal tubules, so it is not clear how susceptible other renal

tubular cell populations are to direct exposure to mercury or what role injury in these segments plays in the overall toxic response in the kidney.

Although intrarenal accumulation of mercury per se would logically seem to be the parameter that one would characterize and expect to correlate with the severity of renal injury induced by mercury, it appears that urinary excretion of mercury correlates much more closely to the severity of renal injury. This is an important observation because it can potentially provide a noninvasive means for assessment of the severity of the acute nephropathy induced by mercury. An additional noninvasive mechanism to monitor exposure to mercury and severity of renal injury induced by mercury is measurement of the urinary excretion of a number of plasma solutes and renal cellular enzymes.

D. Factors That Influence Renal Toxicity of Mercury

As a means to both better understand the mechanism of action of mercury-containing compounds and develop antidotes and treatments for mercury-poisoning, factors, both intracellular and extracellular, have been investigated as tools to alter the disposition and metabolism of mercury. The intracellular content of glutathione or metallothionein can be manipulated by a number of techniques to alter the intracellular distribution of mercury-containing compounds. Similarly, exogenous glutathione or metallothionein can influence the renal accumulation and toxicity of mercury. In addition, therapeutic metal chelators, most notably DMPS and DMSA, have been used clinically as antidotes to either reverse or prevent the toxic effects of mercury-containing compounds.

Physiological or pathological processes can dramatically alter the renal handling of, and cellular responses to, mercury. One such process that has received considerable attention is reduced renal mass and compensatory renal growth. Justification for the study of this model lies in the fact that there is a large population of individuals in our society who have reduced renal mass (caused by a whole host of factors) and these individuals may be at a greater risk of becoming intoxicated by mercury-containing or other nephrotoxic agents. Hence, an understanding of how compensatory renal growth and progressive losses in the number of functioning nephrons modify the handling and toxicity of mercury in the remnant renal tissue is a very relevant and important issue.

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REFERENCES

Adam KR (1951) The effects of dithiols on the distribution of mercury in rabbits. *Br J Pharmacol* **6:**483–491.

Addya A, Chakravarti K, Basu A, Santra M, Haldar S and Chatterjee GC (1984) Effects of mercuric chloride on several scavenging enzymes in rat kidney and influence of vitamin E supplementation. *Acta Vitaminol Enzymol* **6:**103–107.

Agency for Toxic Substance and Disease Registry (1999) *Toxicological Profile for Mercury* US Department of Health and Human Services, Public Health Service, Agency for Toxic Substance and Disease Registry, Bethesda, MD, publication no. TP-93/10.

Aleo MD, Taub ML and Kostyniak PJ (1992) Primary cultures of rabbit renal proximal tubule cells. III. Comparative cytotoxicity of inorganic and organic mercury. *Toxicol Appl Pharmacol* **112:**310–317.

Aleo MD, Taub ML, Olson JR, Nickerson PA and Kostyniak PJ (1987) Cellular uptake and response of primary cultures of rabbit renal proximal tubule cells exposed to mercuric chloride and methylmercury chloride, in *In Vitro Toxicology: Approaches to Validation* (Goldberg AM ed) pp 211–226, Mary Ann Liebert, Inc, New York.

Anner BM and Moosmayer M (1992) Mercury inhibits Na-K-ATPase primarily at the cytosolic side. *Am J Physiol* **262:**F843–F848.

Anner BM, Moosmayer M and Imesch E (1992) Mercury blocks Na-K-ATPase by a ligand-dependent and reversible mechanism. *Am J Physiol* **262:**F830–F836.

Aposhian HV (1983) DMSA and DMPS: Water soluble antidotes for heavy metal poisoning. *Annu Rev Pharmacol Toxicol* **23:**193–215.

Aposhian HV and Aposhian MM (1990) *meso*-2,3-Dimercaptosuccinic acid: Chemical, pharmacological and toxicological properties of an orally effective metal chelating agent. *Annu Rev Pharmacol Toxicol* **30:**279–306.

Ashe WF, Largent EJ, Dutra FR, Hubbard DM and Blackstone M (1953) Behavior of mercury in the animal organism following inhalation. *Arch Ind Hygiene* **7:**19–43.

Aschner M and Clarkson TW (1989) Methyl mercury uptake across bovine brain capillary endothelial cells in vitro: The role of amino acids. *Pharmacol Toxicol* **64:**293–297.

Aschner M, Eberle NB, Goderie S and Kimelberg HK (1990) Methylmercury uptake in rat primary astrocyte cultures: The role of the neutral amino acid transport system. *Brain Res* **521:**221–228.

Aw TY, Wierzbicka G and Jones DP (1991) Oral glutathione increases tissue glutathione *in vivo*. *Chem-Biol Interact* **80:**89–97.

Baggett J McC and Berndt WO (1985) The effect of potassium dichromate and mercuric chloride on urinary excretion and organ and subcellular distribution of [^{203}Hg]mercuric chloride in rats. *Toxicol Lett* **29:**115–121.

Baggett J McC and Berndt WO (1986) The effect of depletion of nonprotein sulfhydryls by diethyl maleate plus buthionine sulfoximine on renal uptake of mercury in the rat. *Toxicol Appl Pharmacol* **83:**556–562.

Ban M and de Ceaurriz J (1988) Probenecid-induced protection against hexachloro-1,3-butadiene and methylmercury toxicity to the mouse kidney. *Toxicol Lett* **40:**71–76.

Barenberg RL, Solomon S, Papper S and Anderson R (1968) Clearance and micropuncture study of renal function in mercuric chloride treated rats. *J Lab Clin Med* **72:**473–484.

Barfuss DW, Robinson MK and Zalups RK (1990) Inorganic mercury transport in the proximal tubule of the rabbit. *J Am Soc Nephrol* **1:**910–917.

Barnes JL, McDowell EM, McNeil JS, Flamenbaum W and Trump BF (1980) Studies on the pathophysiology of acute renal failure. IV. Protective effect of dithiothreitol following administration of mercuric chloride in the rat. *Virchows Arch B Cell Pathol* **32:**201–232.

Bergstrand A, Friberg L, Mendel L and Odeblad E (1959) The localization of subcutaneously administered radioactive mercury in the rat kidney. *J Ultrastruct Res* **3:**238–239.

Berlin M (1963) Accumulation and retention of mercury in the mouse. III. An autoradiographic comparison of methylmercuric dicyandiamide with inorganic mercury. *Arch Environ Health* **6:**610–616.

Berlin M and Gibson S (1963) Renal uptake, excretion, and retention of mercury. I. A study in the rabbit during infusion of mercuric chloride. *Arch Environ Health* **6:**617–625.

Berlin M and Ullberg S (1963a) Accumulation and retention of mercury in the mouse. I. An autoradiographic study after a single intravenous injection of mercuric chloride. *Arch Environ Health* **6:**582–601.

Berlin M and Ullberg S (1963b) Accumulation and retention of mercury in the mouse. II. An autoradiographic comparison of phenylmercuric acetate with inorganic mercury. *Arch Environ Health* **6:**602–609.

Berndt WO, Baggett J McC, Blacker A and Houser M (1985) Renal glutathione and mercury uptake by kidney. *Fundam Appl Toxicol* **5:**832–839.

Bigazzi PE (1988) Autoimmunity induced by chemicals. *Clin Toxicol* **26:**125–156.

Bigazzi PE (1992) Lessons from animal models: The scope of mercury-induced autoimmunity. *Clin Immunol Immunopathol* **65:**81–84.

Bowers MA, Aicher LD, Davis HA and Woods JS (1992) Quantitative determination of porphyrins in rat and human urine and evaluation of urinary porphyrin profiles during mercury and lead exposures. *J Lab Clin Med* **120:**272–281.

Brown DL, Reuhl KR, Bormann S and Little JE (1988) Effects of methyl mercury on the microtubule system of mouse lymphocytes. *Toxicol Appl Pharmacol* **94:**66–75.

Brown JR and Shockley P (1982) Serum albumin: Structure and characterization of its ligand binding sites, in *Lipid-Protein Interactions* (Jost PC and Griffith eds) vol 1, pp 25–68, John Wiley & Sons, Inc, New York.

Buchet JP, Roels H, Bernard A and Lauwreys R (1980) Assessment of renal function of workers exposed to inorganic lead, cadmium or mercury vapor. *J Occup Med* **22:**741–750.

Cannon VT, Barfuss DW and Zalups RK (1998a) The role of γ-glutamyltransferase (γ-GT) on the disappearance of mercury from the lumen of proximal tubular segments perfused with GSH and mercury. *Toxicol Sci* **42:**377.

Cannon VT, Zalups RK and Barfuss DW (1998) Mechanisms involved in the luminal disappearance of GSH_2-Hg, CYS-GLY_2-Hg, AND CYS_2-Hg in the proximal tubule. *J Am Soc Nephrol* **9:**592A.

Cannon VT, Zalups RK and Barfuss DW (2000) Molecular homology and the luminal transport of Hg^{++} in the renal proximal tubule. *J Am Soc Nephrol*, in press.

Cannon VT, Zalups RK and Barfuss DW (1999) Luminal transport of dicysteinylmercury ($(CYS)_2$-Hg) in the rabbit proximal tubule. *Toxicologist* **48:**332.

Cardenas A, Roels H, Bernard AM, Barbon R, Buchet JP, Lauwreys RR, Rosello J, Hotter G, Mutti A, Franchini I, et al. (1993) Markers of early renal changes

induced by industrial pollutants. I. Application to workers exposed to mercury vapour. *Br J Ind Med* **50:**17–27.
Cember H, Gallagher P and Faulkner A (1968) Distribution of mercury among blood fractions and serum proteins. *Am Ind Hyg Assoc J* **29:**233–237.
Chan HM, Satoh M, Zalups RK and Cherian MG (1992) Exogenous metallothionein and renal toxicity of cadmium and mercury in rats. *Toxicology* **76:**15–26.
Chang LW, Ware RA and Desnoyers PA (1973) A histochemical study on some enzymes changes in the kidney, liver and brain after chronic mercury intoxication in the rat. *Food Cos Toxicol* **11:**283–286.
Chavez E and Holguin JA (1988) Mitochondrial calcium release induced by Hg^{2+}. *J Biol Chem* **283:**3582–3587.
Chavez E, Zazueta C, Osornio A, Holguin JA and Miranda ME (1991) Protective behavior of captopril on Hg^{++}-induced toxicity on kidney mitochondria: *In vivo* and *in vitro* experiments. *J Pharmacol Exp Ther* **256:**385–390.
Cherian MG and Clarkson TW (1976) Biochemical changes in rat kidney on exposure to elemental mercury vapor: Effect on biosynthesis of metallothionein. *Chem-Biol Interact* **12:**109–120.
Chung A-S, Maines MD and Reynolds WA (1982) Inhibition of the enzymes of glutathione metabolism by mercuric chloride in the rat kidney: Reversal by selenium. *Biochem Pharmacol* **31:**3093–3100.
Clarkson TW (1972) The pharmacology of mercury compounds. *Annu Rev Pharmacol Toxicol* **12:**375–406.
Clarkson TW (1993) Molecular and ionic mimicry of toxic metals. *Annu Rev Pharmacol Toxicol* **32:**545–571.
Clarkson TW and Magos L (1967) The effect of sodium maleate on the renal disposition and excretion of mercury. *Br J Pharmacol Chemother* **31:**560–567.
Conner EA and Fowler BA (1993) Mechanisms of metal-induced nephropathy, in *Toxicity of the Kidney*, 2nd ed (Hook JB and Goldstein RS eds) pp 437–457, Raven Press, New York.
Cuppage PE, Chiga M and Tate A (1972) Cell cycle studies in the regenerating rat nephron following injury with mercuric chloride. *Lab Invest* **26:**122–126.
Cuppage PE and Tate A (1967) Repair of the nephron following injury with mercuric chloride. *Am J Pathol* **51:**405–429.
Danscher G, Horsted-Bindslev P and Rungby J (1990) Traces of mercury in organs from primates with amalgam fillings. *Exp Mol Pathol* **52:**291–299.
Dantzler WH (1996) Comparative aspects of renal organic anion transport. *Cell Physiol Biochem* **6:**28–38.
Dantzler WH, Evans KK and Wright SH (1995) Kinetics of interactions of para-aminohippurate, probenecid, cysteine conjugates and N-acetylcysteine conjugates with basolateral organic anion transporter in isolated rabbit proximal renal tubules. *J Pharmacol Exp Ther* **272:**663–672.
de Ceaurriz J, Payan JP, Morel G and Brondeau MT (1994) Role of extracellular glutathione and γ-glutamyltranspeptidase in the disposition and kidney toxicity of inorganic mercury in rats. *J Appl Toxicol* **14:**201–206.
Diamond GL (1988) Biological monitoring of urine for exposure to toxic metals, in *Biological Monitoring of Toxic Metals* (Clarkson TW, Friberg L, Nordberg GF and Sager PR eds) pp 515–529, Plenum Publishing Corporation, New York.
Druet P, Druet E, Potdevin F and Sapin C (1978) Immune type glomerulonephritis induced by $HgCl_2$ in the Brown-Norway rat. *Ann Immunol (Institute Pasteur)* **129:**C777–C792.
Dunn JD and Clarkson TW (1980) Does mercury exhalation signal demethylation of methylmercury? *Health Phys* **38:**411–414.
Ellis BG, Price RG and Topham JC (1973) The effect of tubular damage by mercuric chloride on kidney function and some urinary enzymes in the dog. *Chem-Biol Interact* **7:**101–113.
Enestrom S and Hultman P (1984) Immune-mediated glomerulonephritis induced by mercuric chloride in mice. *Experientia* **40:**1234–1240.
Ferrier B, Martin M and Roch-Ramel F (1983) Effects of *p*-aminohippurate and pyrazinoate on the renal excretion of salicylate in the rat: A micropuncture study. *J Pharmacol Exp Ther* **224:**451–458.
Fitzgerald WF and Clarkson TW (1991) Mercury and monomethylmercury: Present and future concerns. *Environ Health Perspectives* **96:**159–166.
Foulkes EC (1974) Excretion and retention of cadmium, zinc, and mercury by rabbit kidney. *Am J Physiol* **227:**1356–1360.
Fowler BA (1972) The morphological effects of dieldrin and methyl mercuric chloride on pars recta segments of the rat kidney proximal tubules. *Am J Pathol* **69:**163–178.
Friberg L (1956) Studies on the accumulation, metabolism and excretion of inorganic mercury (^{203}Hg) after prolonged subcutaneous administration to rats. *Acta Pharmacol Toxicol* **12:**411–427.
Friberg L (1959) Studies on the metabolism of mercuric chloride and methyl mercury dicyandiamide: Experiments on rats given subcutaneous injections with radioactive mercury (^{203}Hg). *AMA Arch Ind Health* **20:**42–49.
Friberg L, Odeblad E and Forssman S (1957) Distribution of 2 mercury compounds in rabbits after a single subcutaneous injection. *AMA Arch Ind Health* **16:**163–168.
Friedman HL (1957) Relationship between chemical structure and biological activity in mercurial compounds. *Ann NY Acad Sci* **65:**461–470.
Fuhr BJ and Rabenstein DL (1973) Nuclear magnetic-resonance studies of solution chemistry of metal-complexes. 9. Binding of cadmium, zinc, lead and mercury by glutathione. *J Am Chem Soc* **95:**6944–6950.
Fukino H, Hirai M, HsuehYM, Moriyasu S and Yamane Y (1986) Mechanism of protection by zinc against mercuric chloride toxicity in rats: Effects of zinc and mercury on glutathione metabolism. *J Toxicol Environ Health* **19:**75–89.
Fukino H, Hirai M, Hsueh YM and Yamane Y (1984) Effect of zinc pretreatment on mercuric chloride-induced lipid peroxidation in the rat kidney. *Toxicol Appl Pharmacol* **73:**395–401.
Gage JC (1964) Distribution and excretion of methyl and phenylmercury salts. *Br J Ind Med* **21:**197–202.
Ganote CE, Reimer KA and Jennings RB (1974) Acute mercuric chloride nephrotoxicity: An electron microscopic and metabolic study. *Lab Invest* **31:**633–647.
Girardi G and Elias MM (1991) Effectiveness of *N*-acetylcysteine in protecting against mercuric chloride-induced nephrotoxicity. *Toxicology* **67:**155–164.
Girardi G and Elias MM (1993) Effect of different renal glutathione levels on renal mercury disposition and excretion in the rat. *Toxicology* **81:**57–67.
Gleason MN, Gosselin RE and Hodge DC (1957) *Clinical Toxicology of Commercial Products* p 154, Williams & Wilkins, Baltimore.
Goering PL, Fisher BR, Chaudhary PP and Dick CA (1992) Relationship between stress protein induction in rat kidney by mercuric chloride and nephrotoxicity. *Toxicol Appl Pharmacol* **113:**184–191.
Gottelli CA, Astolfi E, Cox C, Cernichiari E and Clarkson TW (1985) Early biochemical effects of organic mercury fungicide on infants: "Dose makes the poison." *Science (Wash DC)* **227:**638–640.
Gritzka TL and Trump BF (1968) Renal tubular lesions caused by mercuric chloride: Electron microscopic observations: Degeneration of the pars recta. *Am J Pathol* **52:**1225–1277.
Gstraunthaler G, Pfaller W and Kotanko P (1983) Glutathione depletion and *in vitro* lipid peroxidation in mercury or maleate-induced acute renal failure. *Biochem Pharmacol* **32:**2969–2972.
Hahn LJ, Kloiber R, Leininger RW, Vimy MJ and Lorscheider FL (1990) Whole-body imaging of the distribution of mercury released from dental fillings into monkey tissues. *FASEB J* **4:**3256–3260.
Hahn LJ, Kloiber R, Vimy MJ, Takahashi Y and Lorscheider FL (1989) Dental "silver" tooth fillings: A source of mercury exposure revealed by whole-body image scan and tissue analysis. *FASEB J* **3:**2641–2646.
Hernandez-Pando R, Pedraza-Chaverri J, Orozco-Estevez H, Silva-Serna P, Moreno I, Rondan-Zarate A, Elinos M, Correa-Rotter R and Larriva-Sahd J (1995) Histological and subcellular distribution of 65 and 70 kD heat shock proteins in experimental nephrotoxic injury. *Exp Toxicol Pathol* **47:**501–508.
Houser MT and Berndt WO (1986) The effect of unilateral nephrectomy on the nephrotoxicity of mercuric chloride in the rat. *Toxicol Appl Pharmacol* **83:**506–515.
Houser MT and Berndt WO (1988) Unilateral nephrectomy in the rat: Effects on mercury handling and renal cortical subcellular distribution. *Toxicol Appl Pharmacol* **93:**187–194.
Hultman P and Enestrom S (1986) Localization of mercury in the kidney during experimental acute tubular necrosis studied by the cytochemical silver amplification method. *Br J Exp Pathol* **67:**493–503.
Hultman P and Enestrom S (1992) Dose-response studies in murine mercury-induced autoimmunity and immune-complex disease. *Toxicol Appl Pharmacol* **113:**199–208.
Hultman P, Enestrom S and von Schenck H (1985) Renal handling of inorganic mercury in mice: The early excretion phase following a single intravenous injection of mercuric chloride studied by the silver amplification method. *Virchows Arch [Cell Pathol]* **49:**209–224.
Imesch E, Moosmayer M and Anner BM (1992) Mercury weakens membrane anchoring of Na-K-ATPase. *Am J Physiol* **262:**F837–F842.
Johnson DR (1982) Role of renal cortical sulfhydryl groups in development of mercury-induced renal toxicity. *J Toxicol Environ Health* **9:**119–126.
Jung KY, Uchida S and Endou H (1989) Nephrotoxicity assessment by measuring cellular ATP content. I. Substrate specificities in the maintenance of ATP content in isolated rat nephron segments. *Toxicol Appl Pharmacol* **100:**369–382.
Keppler D, Leier I, Jedlitschky G and Konig J (1998) ATP-dependent transport of glutathione S-conjugates by the multidrug resistance protein MRP1 and its apical isoform MRP2. *Chem-Biol Interact* **111:**153–161.
Kerper LE, Ballatori N and Clarkson TW (1992) Methylmercury transport across the blood brain barrier by an amino acid carrier. *Am J Physiol* **262:**R761–R765.
Kim CY, Watanabe C, Kasanuma Y and Satoh H (1995) Inhibition of gamma-glutamyltranspeptidase decreases renal deposition of mercury after mercury vapor exposure. *Arch Toxicol* **69:**722–724.
Kirschbaum BB (1979) Alanine aminopeptidase excretion after mercuric chloride renal failure. *Biochem Med* **21:**220–225.
Klein R, Herman SP, Bullock BC and Talley FA (1973) Methyl mercury intoxication in rat kidneys. *Arch Pathol* **96:**83–90.
Koropatnick JD and Zalups RK (1997) Effect of non-toxic mercury, zinc or cadmium pretreatment on the capacity of human monocytes to under lipopolysaccharide-induced activation. *Br J Pharmacol* **120:**797–806.
Koropatnick JD and Zalups RK (2000) Effect of toxic and essential metals on cellular responsiveness to cell signals, in *Molecular Biology and Toxicology of Metals* (Zalups RK and Koropatnick DJ eds) Taylor and Francis, London, in press.
Klotzbach JM and Diamond GL (1988) Complexing activity and excretion of 2,3-dimercapto-1-propane sulfonate in rat kidney. *Am J Physiol* **254:**F871–F878.
Kostial K, Kello D and Jugo S (1978) Influence of age on metal metabolism and toxicity. *Environ Health Perspect* **25:**81–86.
Kuwahara M, Gu Y, Ishibashi K, Marumo F and Sasaki S (1997) Mercury-sensitive residues and pore site in AQP3 water channel. *Biochemistry* **36:**13973–13978.
Lash LH and Anders MW (1989) Uptake of nephrotoxic *S*-conjugates by isolated rat renal proximal tubular cells. *J Pharmacol Exp Ther* **248:**531–537.
Lash LH and Jones DP (1983) Transport of glutathione by renal basal-lateral membrane vesicles. *Biochem Biophys Res Commun* **112:**55–60.
Lash LH and Jones DP (1984) Renal glutathione transport: Characteristics of the sodium-dependent system in the basal-lateral membrane. *J Biol Chem* **259:**14508–14514.
Lash LH and Jones DP (1985a) Distribution of oxidized and reduced forms of glutathione and cysteine in rat plasma. *Arch Biochem Biophys* **240:**583–592.
Lash LH and Jones DP (1985b) Uptake of the glutathione conjugate *S*-(1,2-dichlorovinyl)glutathione by renal basal-lateral membrane vesicles and isolated kidney cells. *Mol Pharmacol* **28:**278–282.
Lash LH, Putt DA and Zalups RK (1998) Role of extracellular thiols in uptake and distribution of inorganic mercury in rat renal proximal and distal tubular cells. *J Pharmacol Exp Ther* **285:**1039–1050.
Lash LH, Putt Da and Zalups RK (1999) Influence of exogenous thiols on inorganic

mercury-induced injury in renal proximal and distal tubular cells from normal and uninephrectomized rats. *J Pharmacol Exp Ther* **291:**492–502.
Lash LH and Zalups RK (1992) Mercuric chloride-induced cytotoxicity and compensatory hypertrophy in rat kidney proximal tubular cells. *J Pharmacol Exp Ther* **261:**819–829.
Lash LH and Zalups RK (1994) Activities of enzymes involved in renal cellular glutathione metabolism after uninephrectomy in the rat. *Arch Biochem Biophys* **309:**129–138.
Lau S and Sarkar B (1979) Inorganic mercury (II)-binding components in normal human blood serum. *J Toxicol Environ Health* **5:**907–916.
Levy RI, Weiner IM and Mudge GH (1958) The effects of acid-base balance on diuresis produced by organic and inorganic mercurials. *J Clin Invest* **37:**1016–1018.
Lund B-O, Miller DM and Woods JS (1991) Mercury-induced H_2O_2 production and lipid peroxidation *in vitro* in rat kidney mitochondria. *Biochem Pharmacol* **42 (Suppl):**S181–S187.
Lund B-O, Miller DM and Woods JS (1993) Studies on Hg(II)-induced H_2O_2 formation and oxidative stress *in vivo* and *in vitro* in rat kidney mitochondria. *Biochem Pharmacol* **45:**2017–2024.
Madsen KM (1980) Mercury accumulation in kidney lysosomes of proteinuric rats. *Kidney Int* **18:**445–453.
Madsen KM and Hansen JC (1980) Subcellular distribution of mercury in the rat kidney cortex after exposure to mercuric chloride. *Toxicol Appl Pharmacol* **54:**443–453.
Magos L, Brown AW, Sparrow S, Bailey E, Snowden RT and Skipp WR (1985) The comparative toxicology of ethyl- and methyl-mercury. *Arch Toxicol* **57:**260–267.
Magos L and Butler WH (1976) The kinetics of methylmercury administered repeatedly to rats. *Arch Toxicol* **35:**25–39.
Magos L and Clarkson TW (1977) Renal injury and urinary excretion, in *Handbook of Physiology*, *Section 9, Renal Physiology* (Lee DHK ed) pp 503–512, American Physiological Society, Washington, DC.
Magos L, Peristianis GG, Clarkson TW, Brown A, Preston S and Snowden RT (1981) Comparative study of the sensitivity of male and female rats to methylmercury. *Arch Toxicol* **48:**11–20.
Magos L and Stoychev T (1969) Combined effect of sodium maleate and some thiol compounds on mercury excretion and redistribution in rats. *Br J Pharmacol* **35:**121–126.
Maiorino RM, Dart RC, Carter DE and Aposhian HV (1991) Determination and metabolism of dithiol chelating agents. XII. Metabolism and pharmacokinetics of sodium 2,3-dimercaptopropane-1-sulfonate in humans. *J Pharmacol Exp Ther* **259:**808–814.
Mambourg AM and Raynaud C (1965) Etude a l'aide d'isotopes radioactifs du mecanisme de l'excretion urinaire du mereure chez le lapin. *Revue Fr Etud Clin Biol* **10:**414–418.
McDowell EM, Nagle RB, Zalme RC, McNeil JS, Flamenbaum W and Trump BF (1976) Studies on the pathophysiology of acute renal failure. I. Correlation of ultrastructure and function in the proximal tubule of the rat following administration of mercuric chloride. *Virchows Arch [Cell Pathol]* **22:**173–196.
McNeil SI, Bhatnagar MK and Turner CJ (1988) Combined toxicity of ethanol and methylmercury in rat. *Toxicology* **53:**345–363.
Meyer TW, Scholey JV and Brenner BM (1991) Nephron adaptation to renal injury, in *The Kidney*, 4th ed (Brenner BM and Rector FC Jr eds) pp 1871–1908, WB Saunders, Philadelphia.
Miller DW and Woods JS (1993) Redox activities of mercury-thiol complexes: Implications for mercury induced porphyria and toxicity. *Chem-Biol Interact* **88:**23–35.
Miura K, Inokawa M and Imura N (1984) Effects of methylmercury and some metal ions on microtubule networks in mouse glioma cells and in vitro tubulin polymerization. *Toxicol Appl Pharmacol* **73:**218–231.
Mussini E (1958) Bonds of mercurial diuretics to blood proteins. *Boll Soc Ital Biol Sper* **34:**1588–1590.
Naganuma A, Oda-Urano N, Tanaka T and Imura N (1988) Possible role of hepatic glutathione in transport of methylmercury into mouse kidney. *Biochem Pharmacol* **37:**291–296.
Nath KA, Croatt AJ and Hostetter TH (1990) Oxygen consumption and oxidant stress in surviving nephrons. *Am J Physiol* **258:**F1354–F1362.
Norseth T and Clarkson TW (1970a) Studies on the biotransformation of ^{203}Hg-labeled methylmercury chloride in rats. *Arch Environ Health* **21:**717–727.
Norseth T and Clarkson TW (1970b) Biotransformation of methylmercury salts in the rat studied by specific determination of inorganic mercury. *Biochem Pharmacol* **19:**2775–2783.
Omata S, Sato M, Sakimura K and Sugano H (1980) Time-dependent accumulation of inorganic mercury in subcellular fractions of kidney, liver, and brain or rats exposed to methylmercury. *Arch Toxicol* **44:**231–241.
Parks LD, Zalups RK and Barfuss DW (1998) Heterogeneity of glutathione synthesis and secretion in the proximal tubule of the rabbit. *Am J Physiol* **274:**F924–F931.
Parks LD, Zalups RK and Barfuss DW (2000) Heterogeneity of glutathione transport in the proximal tubule of the rabbit. *J Am Soc Nephrol*, in press.
Piotrowski JK, Trojanowska B, Wisniewska-Knypl JM and Bolanowska W (1974) Mercury binding in the kidney and liver of rats repeatedly exposed to mercuric chloride. *Toxicol Appl Pharmacol* **27:**11–19.
Planas-Bohne F (1977) The effect of mercuric chloride on the excretion of two urinary enzymes in the rat. *Arch Toxicol* **37:**219–225.
Planas-Bohne F (1981) The effect of 2,3-dimercaptopropane-1-sulfonate and dimercaptosuccinic acid on the distribution and excretion of mercury in rats. *Toxicology* **19:**275–278.
Pritchard JB (1988) Coupled transport of *p*-aminohippurate by rat kidney basolateral membrane vesicles. *Am J Physiol* **255:**F597–F604.
Pritchard JB and Miller DS (1993) Mechanisms mediating renal secretion of organic anions and cations. *Physiol Rev* **73:**765–788.
Price RG (1982) Urinary enzymes, nephrotoxicity and renal disease. *Toxicology* **23:**99–134.
Prickett CS, Laug EP and Kunze FM (1950) Distribution of mercury in rats following oral and intravenous administration of mercuric acetate and phenylmercuric acetate. *Proc Soc Exp Biol Med* **73:**585–588.
Rabenstein DL (1989) Metal complexes of glutathione and their biological significance, in *Glutathione: Chemical, Biochemical and Medical Aspects, Vol 3, Coenzymes and Cofactors* (Dophin D, Auramovibc O and Poulson R eds) pp 147–186. Wiley, New York.
Roch-Ramel F, Besseghir K and Murer H (1992) Renal excretion and tubular transport of organic anions and cations, in *Handbook of Physiology, Section 8, Renal Physiology* (Windhager EE ed) pp 2189–2262, Oxford University Press, New York.
Rodier PM, Kates B and Simons R (1988) Mercury localization in mouse kidney over time: Autoradiography versus silver staining. *Toxicol Appl Pharmacol* **257:**235–245.
Rodin AE and Crowson CN (1962) Mercury nephrotoxicity in the rat. I. Factors influencing the localization of tubular lesions. *Am J Pathol* **41:**297–313.
Rogulski J and Angielski S (1963) Effect of maleic acid on the kidney. *Acta Biochem Pol* **10:**133–139.
Roman-Franco AA, Turiello M, Albini B, Ossi E, Milgrom F and Andres GA (1978) Anti-basement membrane antibodies and antigen-antibody complexes in rabbits injected with mercuric chloride. *Clin Immunol Immunopathol* **9:**464–481.
Rothstein A (1970) Sulfhydryl groups in membrane structure and function. *Curr Top Membr Transm* **1:**135–176.
Rothstein A and Hayes AD (1960) The metabolism of mercury in the rat studied by isotope techniques. *J Pharmacol Exp Ther* **130:**166–176.
Ruegg CE, Gandolfi AJ, Nagle RB and Brendel K (1987) Differential patterns of injury to the proximal tubule of renal cortical slices following in vitro exposure to mercuric chloride, potassium dichromate, or hypoxic conditions. *Toxicol Appl Pharmacol* **90:**261–273.
Sager PR and Syversen TL (1984) Differential responses to methylmercury exposure and recovery in neuroblastoma and glioma cells and fibroblasts. *Exp Neurol* **85:**371–382.
Sapin C, Dreut E and Dreut P (1977) Induction of anti-glomerular basement membrane antibodies in the brown Norway rat by mercuric chloride. *Clin Exp Immunol* **28:**173–179.
Schafer JA and Watkins ML (1984) Transport of L-cystine in isolated perfused proximal straight tubules. *Pfluegers Arch* **401:**143–151.
Schaub TP, Kartenbeck J, Konig J, Spring H, Dorsam J, Staehler G, Storkel S, Thon WF and Keppler D (1999) Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubules and in renal cell carcinoma. *J Am Soc Nephrol* **10:**1159–1169.
Schaub TP, Kartenbeck J, Konig J, Vogel O, Witzgall R, Kriz W and Keppler D (1997) Expression of the conjugate export pump encoded by the mrp2 gene in the apical membrane of kidney proximal tubules. *J Am Soc Nephrol* **8:**1213–1221.
Shimomura A, Chonko AM and Grantham JJ (1981) Basis for heterogeneity for para-aminohippurate secretion in rabbit proximal tubules. *Am J Physiol* **241:** F430–F436.
Siegers C-P, Schenke M and Younes M (1987) Influence of cadmium chloride, mercuric chloride, and sodium vanadate on the glutathione-conjugating enzyme system in liver, kidney, and brain of mice. *J Toxicol Environ Health* **22:**141–148.
Silbernagl S (1992) Tubular transport of amino acids and small peptides, in *Handbook of Physiology, Section 8, Renal Physiology* (Windhager EE ed) pp 1938–1976, Oxford University Press, New York.
Smith JC, Allen PV, Turner MD, Most B, Fisher HL and Hall LL (1994) The kinetics of intravenously administered methyl mercury in man. *Toxicol Appl Pharmacol* **128:**251–256.
Smith MA, Acosta D and Bruckner JV (1986) Development of a primary culture system of rat kidney cortical cells to evaluate the nephrotoxicity of xenobiotics. *Food Chem Toxicol* **24:**551–556.
Smith MW, Ambudkar IS, Phelps PC, Regec AL and Trump BF (1987) $HgCl_2$-induced changes in cytosolic Ca^{2+} of cultured rabbit renal tubular cells. *Biochim Biophys Acta* **931:**130–142.
Stewart JR and Diamond GL (1988) *In vivo* tubular secretion and metabolism of the disulfide of 2,3-dimercaptopropane-1-sulfonate. *Drug Metab Dispos* **16:**189–195.
Stonard MD, Chater BV, Duffield DB, Nevitt AL, O'Sullivan JJ and Steele GT (1983) An evaluation of renal function in workers occupationally exposed to mercury vapor. *Int Arch Occup Environ Health* **52:**177–189.
Stroo WE and Hook JB (1977) Enzymes of renal origin in urine as indicators of nephrotoxicity. *Toxicol Appl Pharmacol* **39:**423–434.
Swensson A and Ulfvarson U (1968) Distribution and excretion of mercury compounds in rats over a long period after a single injection. *Acta Pharmacol Toxicol* **26:**273–283.
Tanaka T, Naganuma A and Imura N (1990) Role of γ-glutamyltranspeptidase in renal uptake and toxicity of inorganic mercury in mice. *Toxicology* **60:**187–198.
Tanaka T, Naganuma A and Imura N (1992) Routes for renal transport of methylmercury in mice. *Eur J Pharmacol* **228:**9–14.
Tanaka-Kagawa T, Naganuma A and Imura N (1993) Tubular secretion and reabsorption of mercury compounds in mouse kidney. *J Pharmacol Exp Ther* **264:**776–782.
Taugner R, Winkel K and Iravani J (1966) Zur Lokalization der Sublimatanreicherung in der Rattenneire. *Virchows Arch Pathol Anat Physiol* **340:**369–383.
Troen P, Kaufman SA and Katz KH (1951) Mercuric bichloride poisoning. *New Engl J Med* **244:**459–463.
Trojanowska B, Piotrowski JK and Szendzikowski S (1971) The influence of thioacetamide on the excretion of mercury in rats. *Toxicol Appl Pharmacol* **18:**374–386.
Ullrich KJ, Rumrich G, Fasold G and Kloss S (1987a) Contraluminal para-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. I. Kinetics, influence of cations, anions, and capillary preperfusion. *Pfluegers Arch* **409:**229–235.
Ullrich KJ, Rumrich G, Fasold G and Kloss S (1987b) Contraluminal para-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. II. Specificity: Aliphatiic dicarboxylic acids. *Pfluegers Arch* **408:**38–45.

Verity MA and Brown WJ (1970) Hg^{2+}-induced kidney necrosis: Subcellular localization and structure-linked lysosomal enzyme changes. *Am J Pathol* **61:**57–74.
Verkman AS (1992) Water channels in cell membranes. *Annu Rev Physiol* **54:**97–108.
Verkman AS (1999) Lessons on renal physiology from transgenic mice lacking aquaporin water channels. *J Am Soc Nephrol* **10:**1126–1135.
Vogel DG, Margolis RL and Mottet NK (1985) The effects of methyl mercury binding to microtubules. *Toxicol Appl Pharmacol* **80:**473–486.
Vostal J (1966) Study of the renal excretatory mechanisms of heavy metals. Proceedings of the 15th International Congress on Occupational Health, Vienna **3:**61–64.
Weening JJ, Hoedemaeker PJ and Bakker WW (1981) Immuno-regulation and anti-nuclear antibodies in mercury-induced glomerulopathy in the rat. *Clin Exp Immunol* **45:**64–71.
Weinberg JM, Harding PG and Humes HD (1982a) Mitochondrial bioenergetics during the initiation of mercuric chloride-induced renal injury. I. Direct effects of *in vitro* mercuric chloride on renal cortical mitochondrial function. *J Biol Chem* **257:**60–67.
Weinberg JM, Harding PG and Humes HD (1982b) Mitochondrial bioenergetics during the initiation of mercuric chloride-induced renal injury. II. Functional alterations of renal cortical mitochondria isolated after mercuric chloride treatment. *J Biol Chem* **257:**68–74.
Woods JS, Calas CA and Aicher LD (1990b) Stimulation of porphyrinogen oxidation by mercuric ion: Promotion of oxidation from the interaction of mercuric ion, glutathione, and mitochondria-generated hydrogen peroxide. *Mol Pharmacol* **38:**261–266.
Woods JS, Calas CA, Aicher LD, Robinson BH and Mailer C (1990a) Stimulation of porphyrinogen oxidation by mercuric ion. I. Evidence of free radical formation in the presence of thiols and hydrogen peroxide. *Mol Pharmacol* **38:**253–260.
Woods JS, Davis HA and Baer RP (1992) Enhancement of γ-glutamylcysteine synthetase mRNA in rat kidney by methyl mercury. *Arch Biochem Biophys* **296:**350–353.
Woods JS, Martin MD, Naleway CA and Echeverria D (1993) Urinary porphyrin profiles as a biomarker of mercury exposure: Studies on dentists with occupational exposure to mercury vapor. *J Toxicol Environ Health* **40:**239–250.
World Health Organization (1991) *Environmental Health Criteria 118: Inorganic Mercury*. World Health Organization, Geneva.
Zalme RC, McDowell FM, Nagle RB, McNeil JS, Flamenbaum W and Trump BF (1976) Studies on the pathophysiology of acute renal failure. II. A histochemical study of the proximal tubule of the rat following administration of mercuric chloride. *Virchows Arch [Cell Pathol]* **22:**197–216.
Zalups RK (1989) Effect of dietary K^+ and 75% nephrectomy on the morphology of principal cells in CCDs. *Am J Physiol* **257:**F387—F396.
Zalups RK (1991a) Autometallographic localization of inorganic mercury in the kidneys of rats: Effect of unilateral nephrectomy and compensatory renal growth. *Exp Mol Pathol* **54:**10–21.
Zalups RK (1991b) Method for studying the *in vivo* accumulation of inorganic mercury in segments of the nephron in the kidneys of rats treated with mercuric chloride. *J Pharmacol Methods* **26:**89–104.
Zalups RK (1991c) Renal accumulation and intrarenal distribution of inorganic mercury in the rabbit: Effect of unilateral nephrectomy and dose of mercuric chloride. *J Toxicol Environ Health* **33:**213–228.
Zalups RK (1993a) Early aspects of the intrarenal distribution of mercury after the intravenous administration of mercuric chloride. *Toxicology* **79:**215–228.
Zalups RK (1993b) Influence of 2,3-dimercaptopropane-1-sulfonate (DMPS) and meso-2,3-dimercaptosuccinic acid (DMSA) on the renal disposition of mercury in normal and uninephrectomized rats exposed to inorganic mercury. *J Pharmacol Exp Ther* **267:**791–800.
Zalups RK (1995) Organic anion transport and action of γ-glutamyltranspeptidase in kidney linked mechanistically to renal tubular uptake of inorganic mercury. *Toxicol Appl Pharmacol* **132:**289–298.
Zalups RK (1996) Enhanced renal outer medullary uptake of mercury associated with uninephrectomy: Implication of a luminal mechanism. *J Toxicol Environ Health* **50:**173–194.
Zalups RK (1997) Enhanced renal outer medullary uptake of mercury associated with uninephrectomy: Implication of a luminal mechanism. *J Toxicol Environ Health* **50:**173–194.
Zalups RK (1998a) Intestinal handling of mercury in the rat: Implication of intestinal secretion of inorganic mercury following biliary ligation or cannulation. *J Toxicol Environ Health* **53:**615–636.
Zalups RK (1998b) Basolateral uptake of inorganic mercury in the kidney. *Toxicol Appl Pharmacol* **150:**1–8.
Zalups RK (1998c) Basolateral uptake of mercuric conjugates of *N*-acetylcysteine and cysteine in the kidney involves the organic anion transport system. *J Toxicol Environ Health* **54:**101–117.
Zalups RK and Barfuss DW (1990) Accumulation of inorganic mercury along the renal proximal tubule of the rabbit. *Toxicol Appl Pharmacol* **106:**245–253.
Zalups RK and Barfuss DW (1993a) Transport and toxicity of methylmercury along the proximal tubule of the rabbit. *Toxicol Appl Pharmacol* **121:**176–185.
Zalups RK and Barfuss DW (1993b) Intrarenal distribution of inorganic mercury and albumin after coadministration. *Toxicol Appl Pharmacol* **40:**77–103.
Zalups RK and Barfuss DW (1995a) Pretreatment with *p*-aminohippurate inhibits the renal uptake and accumulation of injected inorganic mercury in the rat. *Toxicology* **103:**23–35.
Zalups RK and Barfuss DW (1995b) Accumulation and handling of inorganic mercury in the kidney after co-administration with glutathione. *J Toxicol Environ Health* **44:**385–399.
Zalups RK and Barfuss DW (1995c) Renal disposition of mercury in rats after intravenous injection of inorganic mercury and cysteine. *J Toxicol Environ Health* **44:**401–413.
Zalups RK and Barfuss DW (1996a) Diversion or prevention of biliary outflow from the liver diminishes the renal uptake of injected inorganic mercury. *Drug Metab Dispos* **24:**480–486.
Zalups RK and Barfuss DW (1996b) Nephrotoxicity of inorganic mercury coadministered with L-cysteine. *Toxicology* **109:**15–29.
Zalups RK and Barfuss DW (1998a) Small aliphatic dicarboxylic acids inhibit renal uptake of administered mercury. *Toxicol Appl Pharmacol* **148:**183–193.
Zalups RK and Barfuss DW (1998b) Participation of mercuric conjugates of cysteine, homocysteine, and *N*-acetylcysteine in mechanisms involved in the renal tubular uptake of inorganic mercury. *J Am Soc Nephrol* **9:**551–561.
Zalups RK, Barfuss DW and Kostyniak PJ (1992) Altered intrarenal accumulation of mercury in uninephrectomized rats treated with methylmercury chloride. *Toxicol Appl Pharmacol* **115:**174–182.
Zalups RK, Barfuss DW and Lash LH (1999a) Disposition of inorganic mercury following biliary obstruction and chemically-induced glutathione depletion: Dispositional changes 1 h after the intravenous administration of mercuric chloride. *Toxicol Appl Pharmacol* **154:**135–144.
Zalups RK, Barfuss DW and Lash LH (1999b) Effects of biliary ligation and modulation of GSH-status on the renal and hepatic disposition of inorganic mercury in rats. *Toxicologist* **48:**330–331.
Zalups RK, Barfuss DW and Lash LH (1999c) Relationships between alterations in glutathione metabolism and the disposition of inorganic mercury in rats: Effects of biliary ligation and chemically induced modulation of glutathione status. *Chem-Biol Interact* **123:**171–195.
Zalups RK and Cherian MG (1992a) Renal metallothionein metabolism after a reduction of renal mass. I. Effect of unilateral nephrectomy and compensatory renal growth on basal and metal-induced renal metallothionein metabolism. *Toxicology* **71:**83–102.
Zalups RK and Cherian MG (1992b) Renal metallothionein metabolism after a reduction of renal mass. II. Effect of zinc pretreatment on the renal toxicity and intrarenal accumulation of inorganic mercury. *Toxicology* **71:**103–117.
Zalups RK, Cherian MG and Barfuss DW (1993a) Mercury-metallothionein and the renal accumulation and handling of mercury. *Toxicology* **83:**61–78.
Zalups RK, Cox C and Diamond GL (1988) Histological and urinalysis assessment of nephrotoxicity induced by mercuric chloride in normal and uninephrectomized rats, in *Biological Monitoring of Toxic Metals* (Clarkson TW, Friberg L, Nordberg GF and Sager PR eds) pp 531–545, Plenum Publishing Corporation, New York.
Zalups RK and Diamond GL (1987a) Intrarenal distribution of mercury in the rat: Effect of administered dose of mercuric chloride. *Bull Environ Contam Toxicol* **38:**67–72.
Zalups RK and Diamond GL (1987b) Mercuric chloride-induced nephrotoxicity in the rat following unilateral nephrectomy and compensatory renal growth. *Virchows Arch B [Cell Pathol]* **53:**336–346.
Zalups RK, Frazier J and Koropatnick J (1995) Enhanced transcription of metallothionein genes in the kidney of the rat: Effect of uninephrectomy and compensatory renal growth. *Am J Physiol* **268:**F643–F650.
Zalups RK, Gelein RM and Cernichiari E (1991b) DMPS as a rescue agent for the nephropathy induced by mercuric chloride. *J Pharmacol Exp Ther* **256:**1–10.
Zalups RK and Henderson DA (1992) Cellular morphology in outer medullary collecting duct: Effect of 75% nephrectomy and K^+ depletion. *Am J Physiol* **263:**F1119–F1127.
Zalups RK, Klotzbach JM and Diamond GL (1987) Enhanced accumulation of inorganic mercury in renal outer medulla after unilateral nephrectomy. *Toxicol Appl Pharmacol* **89:**226–236.
Zalups RK, Knutson KL and Schnellmann RG (1993b) *In vitro* analysis of the accumulation and toxicity of inorganic mercury in segments of the proximal tubule isolated from the rabbit kidney. *Toxicol Appl Pharmacol* **119:**221–227.
Zalups RK and Lash LH (1990) Effects of uninephrectomy and mercuric chloride on renal glutathione homeostasis. *J Pharmacol Exp Ther* **254:**962–970.
Zalups RK and Lash LH (1994) Advances in understanding the renal transport and toxicity of mercury. *J Toxicol Environ Health* **42:**1–44.
Zalups RK and Lash LH (1997a) Binding of mercury in renal brush-border and basolateral membrane-vesicles: Implication of a cysteine conjugate of mercury involved in the luminal uptake of inorganic mercury. *Biochem Pharmacol* **53:**1889–1900.
Zalups RK and Lash LH (1997b) Depletion of glutathione in the kidney and the renal disposition of administered inorganic mercury. *Drug Metab Dispos* **25:**516–523.
Zalups RK and Minor KH (1995) Luminal and basolateral mechanisms involved in the renal tubular uptake of inorganic mercury. *J Toxicol Environ Health* **46:**73–100.
Zalups RK, Parks L, Cannon VT and Barfuss DW (1998) Mechanisms of action of 2,3-dimercaptopropane-1-sulfonate and the transport, disposition, and toxicity of inorganic mercury in isolated perfused segments of rabbit proximal tubules. *Mol Pharmacol* **54:**353–363.
Zalups RK, Robinson MK and Barfuss DW (1991a) Factors affecting inorganic mercury transport and toxicity in the isolated perfused proximal tubule. *J Am Soc Nephrol* **2:**866–878.
Zalups RK, Stanton BA, Wade JB and Giebisch G (1985) Structural adaptation in initial collecting tubule following reduction in renal mass. *Kidney Int* **27:**636–642.
Zalups RK and Veltman JC (1988) Renal glutathione homeostasis in compensatory renal growth. *Life Sci* **42:**2171–2176.