0022-3565/98/2853-1039$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Vol. 285, No. 3

JPET 285:1039–1050, 1998 *Printed in U.S.A.*

Role of Extracellular Thiols in Accumulation and Distribution of Inorganic Mercury in Rat Renal Proximal and Distal Tubular Cells[1]

LAWRENCE H. LASH, DAVID A. PUTT and RUDOLFS K. ZALUPS

Department of Pharmacology, Wayne State University School of Medicine, Detroit, Michigan (L.H.L., D.A.P.), and Division of Basic Medical Sciences, Mercer University, School of Medicine, Macon, Georgia (R.K.Z.)

Accepted for publication February 4, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

Distribution of inorganic mercury (Hg) into both acid-soluble and protein-bound fractions of proximal tubular (PT) cells from the rat increased with increasing concentrations of Hg up to 10 μM. Little correlation was found between subcellular distribution of Hg and dose in distal tubular (DT) cells. Cellular accumulation of Hg was rapid, reaching equilibrium values by 10 to 15 min. Cellular content of Hg was significantly higher in PT cells than in DT cells at 1 μM Hg. To assess the effect of extracellular thiols on the intracellular accumulation of Hg, PT and DT cells were coincubated with Hg and cysteine, glutathione (GSH), bovine serum albumin (BSA) or 2,3-dimercapto-1-propanesulfonic acid (DMPS) in a 4:1 thiol:Hg molar ratio. Coexposure with Hg and cysteine increased intracellular accumulation of Hg in PT cells at 0.1 μM Hg relative to exposure to Hg alone, consistent with an Hg-cysteine conjugate being a transport form of Hg. In contrast, coexposure with Hg and BSA or DMPS markedly decreased accumulation of Hg relative to cells exposed to Hg alone in both cell types. Coexposure with Hg and GSH also decreased accumulation of Hg relative to exposure to Hg alone, but the decrease was less than coexposure with either BSA or DMPS, suggesting that either an Hg-GSH complex may be a transport form or that some of the Hg-GSH complexes were degraded to Hg-cysteine by the action of brush-border membrane enzymes. These results demonstrate that extracellular thiols markedly alter the renal accumulation of Hg and suggest that some Hg-thiol conjugates may be important physiological transport forms of Hg in the kidney.

Hg is a potent and specific nephrotoxicant *in vivo* that accumulates predominantly in the kidneys (Zalups, 1993a) and selectively in PT cells (Zalups, 1991a, 1991b). Intrarenal content of Hg correlates with the severity of Hg-induced nephropathy or cellular injury only up to the point where injury occurs (Bohets *et al*., 1995; Burton *et al*., 1995; Girardi and Elias, 1991; Houser *et al*., 1992; Johnson, 1982; Zalups and Diamond, 1987; Zalups and Lash, 1990; Zalups *et al*., 1987). Hence, delineation of mechanisms that determine transport and intracellular accumulation of Hg in the target cells within the kidney is critical to understanding Hg-induced nephropathy.

Study and characterization of Hg transport are difficult because of the high binding affinity of Hg toward low-molecular weight thiols, such as GSH and Cys (Rabenstein, 1989) or toward protein sulfhydryl groups, such as those on serum albumin. There is evidence that most of the Hg in plasma is bound to serum albumin (Friedman, 1957; Cember *et al*., 1968; Foulkes, 1974; Lau and Sarkar, 1979). There are three potential mechanisms by which the mercuric ion in Hg-albumin complexes in plasma can be taken up by the kidneys: (1) exchange of the albumin ligand with plasma GSH or Cys and transport of the Hg-GSH or Hg-Cys complexes into renal cells, (2) exchange of the albumin ligand with other protein sulfhydryl groups, such as those on cellular plasma membranes, or (3) endocytosis of Hg with filtered albumin. However, because there is a significant amount of free GSH and Cys in plasma (Lash and Jones, 1985a), Hg bound to these low-molecular-weight thiols are additional forms by which Hg may be translocated to the kidneys. Due to the lability of Hg-S bonds in biological fluids (Oram *et al*., 1996; Rabenstein, 1989), ligand exchange is likely to be an important component of the handling of Hg complexes by the kidneys.

Several studies have provided evidence for a role for exogenous thiols or sulfhydryl-containing compounds in the renal uptake and accumulation of Hg. Studies in isolated PT segments from rabbit kidney (Zalups *et al*., 1993) demonstrated

Received for publication September 16, 1997.
[1] This work was supported by National Institute of Environmental Health Sciences Grant ES05157 to R.K.Z. and L.H.L. L.H.L. is a recipient of a Research Career Development Award from the National Institute of Diabetes and Digestive and Kidney Diseases (Grant K04-DK02090).

ABBREVIATIONS: BSA, bovine serum albumin; Cys, cysteine; DT, Distal tubular; DMPS, 2,3-dimercapto-1-propanesulfonic acid; GGT, γ-glutamyltransferase; GSH, glutathione; Hg, inorganic mercury; PAH, *p*-aminohippurate; PT, proximal tubular; TCA, trichloroacetic acid.

protection from injury induced by exposure to 10 μM Hg by the addition of 40 μM GSH, Cys or BSA to the extracellular medium. This protection was associated with decreased uptake of Hg. In two *in vivo* studies, however, the simultaneous addition of GSH or Cys with Hg produced elevations in the renal concentration of Hg relative to animals that were given Hg alone (Zalups and Barfuss, 1995a, 1995b). There is greater association of Hg with isolated brush-border membrane vesicles from rat renal cortex when they are exposed to Hg-Cys complexes compared with mercuric complexes with other thiols (Zalups and Lash, 1997), and coadministration to rats of low, nephrotoxic doses of Hg with Cys resulted in a significant increase in the content of Hg in renal cortex and outer stripe of the outer medulla compared with that in rats administered Hg alone (Zalups and Barfuss, 1996). Hence, it appears that an Hg-Cys complex may be a transport form of Hg into renal PT cells. By contrast, the synthetic dithiol and clinically used, heavy metal chelator DMPS binds Hg with high affinity, significantly reduces renal accumulation and/or the renal burden of Hg and protects both *in vivo* and *in vitro* from Hg-induced renal injury (Zalups, 1993b; Zalups *et al*., 1991a). Current evidence from studies in renal brush-border and basolateral membrane vesicles (Zalups and Lash, 1997) indicates that complexes of Hg and DMPS are poorly, if at all, transported. This contrasts with free DMPS, which is avidly transported into PT cells (Klotzbach and Diamond, 1988).

The potential role of both intracellular and extracellular GSH in Hg uptake and intracellular accumulation is even less clear. Johnson (1982) decreased total renal nonprotein sulfhydryl content with diethyl maleate pretreatment and observed a decrease in renal accumulation of Hg. Rats that have undergone uninephrectomy and compensatory renal growth exhibit both increased GSH content in the renal outer stripe of the outer medulla and increased accumulation of Hg (both total content and content normalized to either tissue weight or protein) when administered Hg (Zalups and Lash, 1990). In contrast, Girardi and Elias (1991, 1993) and Houser *et al*. (1992) observed an inverse relationship between renal contents of GSH and Hg.

Interpretation of findings on the effects of extracellular GSH are complicated by the potential degradation of GSH or Hg-GSH complexes to Cys or Hg-Cys complexes, respectively, by GGT and dipeptidases on renal brush-border membranes. Inhibition of GGT in mice (Tanaka *et al*., 1990; Tanaka-Kagawa *et al*., 1993) or rats (Baggett and Berndt, 1986; Berndt *et al*., 1985; de Ceaurriz *et al*., 1994; Zalups, 1995) with acivicin [L-(αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid] reduced renal accumulation of Hg compared with animals that were not pretreated with acivicin. Hence, a portion of renal uptake and accumulation of Hg occurs by a GGT-dependent mechanism.

Based on the above-mentioned studies, we hypothesize that under appropriate conditions of time and Hg and/or thiol concentrations, extracellular thiols can alter significantly the intracellular accumulation of Hg in renal epithelial cells. We hypothesize further that coexposure with Hg and GSH or Cys can facilitate accumulation of Hg. To test these hypotheses, we investigated the subcellular distribution of Hg and the influence of extracellular thiols on the accumulation of Hg in PT and DT cells from rat kidney, which represent a target and a nontarget renal cell population, respectively. The results demonstrate differences between PT and DT cells in the distribution of cell-associated Hg into protein-bound and soluble fractions and effects of extracellular thiols on cellular accumulation of Hg, supporting the hypothesis that complexes of Hg with GSH or Cys may be physiological transport forms of Hg. A preliminary report of this work has been presented (Lash *et al*., 1997).

Experimental Procedures

Materials. Percoll, collagenase (type I), BSA (fraction V) and DMPS were purchased from Sigma Chemical (St. Louis, MO). $^{203}HgCl_2$ (specific activity, 2.3 mCi/mg) was purchased from Buffalo Materials (Buffalo, NY). All other chemicals were of the highest purity available and were purchased from commercial sources.

Isolation of rat renal PT and DT cells. Renal cortical cells were isolated from the kidneys of male Sprague-Dawley rats (Harlan, Indianapolis, IN; 200–300 g) by collagenase perfusion (Jones *et al*., 1979). Animals were housed in the Wayne State University vivarium, were allowed access to food and water *ad libitum* and were kept in a room on a 12-hr light/dark cycle. Before surgery, rats were anesthetized with intraperitoneal injections of sodium pentobarbital (50 mg/kg b.wt.). Enriched populations of PT and DT cells were then obtained by Percoll density-gradient centrifugation of cortical cells (Lash and Tokarz, 1989). Briefly, cortical cells (5 ml, 5–8 × 10^6 cells/ml) were layered on 35 ml of 45% (v/v) isosmotic Percoll solution in 50-ml polycarbonate centrifuge tubes and were centrifuged at 4°C for 30 min at 20,000 × *g* in a Sorvall RC2B centrifuge in an SS34 rotor. Fractions were collected, and cell types of origin were identified by the use of marker enzymes and cell type-specific respiratory responses (Lash and Tokarz, 1989). Based on enzymology and morphology, the renal PT cell preparation contains cells derived from both convoluted and straight segments and is estimated to be ≥97% pure; the renal DT cell preparation contains cells derived from the distal convoluted tubule, the cortical collecting duct and connecting tubules but not the thick ascending limbs and is estimated to have <10% contamination from PT cells, with a purity of ~88%.

Before incubations, cells were diluted 5-fold with Krebs-Henseleit buffer, pH 7.4, containing 25 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid and metabolic substrates (5 mM glucose, 5 mM glutamine), washed to remove Percoll and resuspended in fresh buffer at a concentration of 1.2 × 10^6 cells/ml. Cell concentrations were determined in the presence of 0.2% (w/v) trypan blue in a hemacytometer, and cell viability was estimated by either trypan blue exclusion or the release of lactate dehydrogenase activity from the cells (Lash and Tokarz, 1989). All buffers were equilibrated with 95% O_2/5% CO_2, and cells were stored on ice in 25-ml polyethylene Erlenmeyer flasks until used. Incubations were performed in 1.5-ml polyethylene microcentrifuge tubes in a 37°C Dubnoff metabolic shaking incubator (60 cycles/min).

Methods to study distribution of cell-associated Hg. Due to the limitations in our ability to differentiate between transport of Hg into the soluble fraction of cells and direct binding of Hg to cellular plasma membranes or transport followed by binding to cellular plasma or intracellular membranes or intracellular macromolecules, we refer to Hg recovered in cell extracts as "cell-associated" and the process by which this occurs as "accumulation."

Renal PT and DT cells were incubated with $^{203}HgCl_2$ (0.5, 1, 10 and 100 μM; 0.05 μCi per 0.5-ml sample plus cold $HgCl_2$ to the indicated concentration) for 1, 5, 10 or 60 min. At indicated times, aliquots of cells (0.5 ml) were layered on 1 ml of 20% (v/v) Percoll in saline in 1.5-ml microcentrifuge tubes to separate cellular contents from extracellular media. Two fractions were obtained: a supernatant and a cell pellet. The supernatant fraction (1.5 ml), which is equivalent to extracellular media, was removed for gamma counting. The cell pellets were resuspended in 0.5 ml of saline, 0.1 ml of 30% (w/v) TCA was added, the suspension was mixed and the tubes were centrifuged to separate the TCA-soluble (transported Hg that re-

mains unbound or free within the cell) and TCA pellet (membrane- or protein-bound Hg) fractions.

Effect of extracellular thiols on cellular accumulation of Hg. Incubation mixtures (1.0 ml) contained 0.75 ml of appropriate $HgCl_2$ stock solution containing 0.05 μCi of $^{203}HgCl_2$ and 0.25 ml of cells (1.2×10^6 cells/ml) and were placed in 1.5-ml microcentrifuge tubes. Final concentrations of Hg were 0.1, 1, and 5 μM because previous studies (Lash and Zalups, 1992) showed that these concentrations of Hg were below the threshold concentration that produced PT cellular injury. Mixtures were incubated at 37°C with shaking in a Dubnoff water bath for 1, 5, 10 or 60 min. At indicated times, Hg accumulation was stopped by centrifugation for 30 sec at $13,000 \times g$. Supernatants were transferred to 2.0-ml cryovials with a 1.0-ml Hamilton gastight syringe. The syringe was rinsed with 0.5 ml of saline, and the rinse was added to the supernatant (total volume, 1.5 ml). Supernatants and cell pellets were then counted in a gamma counter.

To assess the effects of extracellular thiols on cellular accumulation of Hg, cells were incubated simultaneously with Hg and a 4-fold molar excess of BSA, DMPS, GSH or Cys. This excess of thiols was used to ensure that all Hg ions were bound to a ligand. Data are presented in two formats: First, data for the amount of Hg in extracellular supernatants and cell pellets are expressed in pmol; second, data on the effects of thiols on Hg accumulation are expressed as percent of Hg alone to make a direct comparison of the effects of the four thiols easier. For coexposures with Cys, studies were performed with only 0.1 and 1 μM Hg due to limitations in the supply of radiolabel.

Determination of content of Hg in samples. The radioactivity of ^{203}Hg in samples of cells or cell extracts was determined by counting the samples in a 1282 Compugamma CS deep-well gamma spectrometer (Pharmacia-LKB, Gaithersburg, MD) operating at a counting efficiency of 50% for ^{203}Hg. The actual content of Hg in each sample was calculated by dividing the radioactivity of ^{203}Hg in the sample (dpm) by the specific activity of ^{203}Hg in the stock solution (dpm/nmol). The amount of Hg in each sample was then expressed as pmol/10^6 cells.

Statistical analyses. Results are expressed as the mean ± S.E. of measurements from the indicated number of separate cell preparations. Significant differences among selected means were first assessed by a one-way or two-way analysis of variance. When significant F values were obtained with the analysis of variance, the Fisher's protected least significant difference t test was performed to determine which means were significantly different from each other with two-tail probabilities of $<.05$ considered significant. For figures 1 through 3, results of statistical comparisons are described in the legends for clarity. Otherwise, significant differences are indicated on the figures or in the tables.

Results

Cellular distribution of Hg in renal PT and DT cells. Isolated PT and DT cells were incubated with 0.5, 1, 10 or 100 μM Hg for up to 60 min, and cells were centrifuged through Percoll to separate intracellular from extracellular contents. Cellular contents were then fractionated into TCA-soluble and TCA-pellet fractions to assess the time and concentration dependence of the distribution of Hg into protein-free and protein-bound fractions (figs. 1-3). The percentage of Hg found in the supernatant fraction (extracellular medium) ranged from ~60% to 85% of total cpm over the 60-min time course (fig. 1). Recovery of Hg in the supernatant fraction was nearly maximal by the earliest time point, indicating rapid extraction of Hg from the extracellular medium.

Increased total cpm were recovered in the protein-bound fraction (TCA pellet) with increasing time and concentration of Hg up to 10 μM Hg (fig. 2). A more consistent increase in the fraction of Hg in the TCA-pellet fraction was seen in the PT cells, where the amount of Hg increased from ~12% to 32% within 10 min. In the DT cells, the maximal percent of Hg recovered was somewhat lower, at ~25%. With the 100 μM concentration of Hg, the percent of total cpm recovered decreased to ~12% and 6% in PT cells and DT cells, respectively.

The percentage of total cpm recovered in the protein-free fraction of cells (TCA-soluble) exhibited a similar pattern of distribution as in the TCA-pellet fraction, but somewhat smaller increases occurred with increasing Hg concentration and time (fig. 3). The major difference, however, was that markedly higher amounts of Hg were recovered in the TCA-soluble fraction of PT cells after exposure to 100 μM Hg than after exposure to 10 μM Hg, which is the reverse pattern of that seen in the TCA pellet fraction. There was no effect of time or concentration of Hg on the recovery of total cpm in the TCA pellet fraction of DT cells.

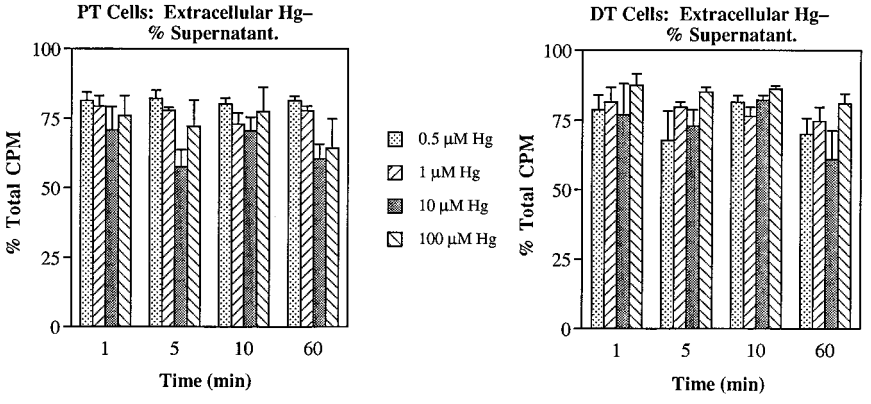

Fig. 1. Distribution of Hg: Extracellular Hg-supernatant. PT (A) and DT cells (B) from rat kidneys (1.2×10^6 cells/ml) were incubated with $^{203}HgCl_2$ (0.5, 1, 10 or 100 μM; 0.05 μCi/0.5-ml sample plus cold $HgCl_2$ to the indicated concentration) for 1, 5, 10 and 60 min. At the indicated times, aliquots of cells (0.5 ml) were layered onto 1 ml of 20% (v/v) Percoll in 1.5-ml microcentrifuge tubes and centrifuged at $13,000 \times g$ for 30 sec. The extracellular supernatants (1.5 ml) were removed for gamma counting. The percent of total cpm from PT and DT cells that was recovered in the extracellular supernatant is plotted versus incubation time. Results are mean ± S.E. of measurements from four separate cell preparations. Statistical analyses: The following pairs ([Hg]/time) were significantly different ($P < .05$) from each other: For PT cells, 0.5/5 and 10/5, 0.5/10 and 10/10, 0.5/60 and 10/60, 0.5/60 and 100/60, 1/5 and 10/5, 1/10 and 10/10, 1/60 and 10/60, 10/5 and 100/5. For DT cells, 0.5/10 and 100/10, 10/5 and 10/60, 10/60 and 100/60. For PT cells *vs*. DT cells, 10/5.

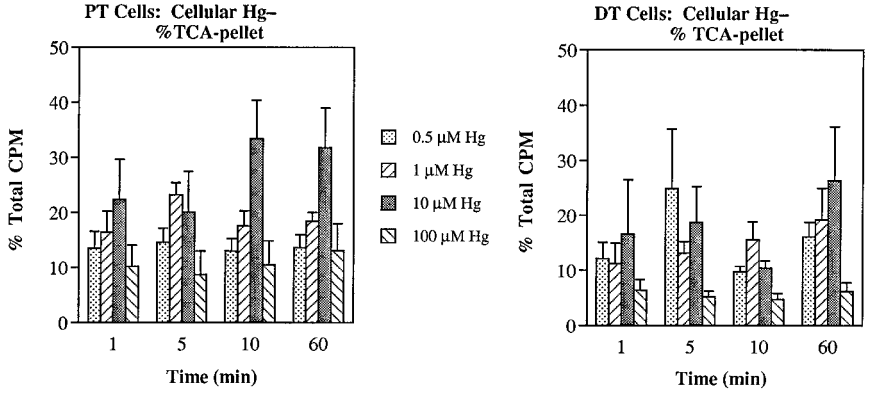

Fig. 2. Distribution of Hg: Cellular Hg-TCA pellet. PT and DT cells from rat kidneys (1.2×10^6 cells/ml) were incubated with $^{203}HgCl_2$ and processed as described in the legend to figure 1. The cell pellets were resuspended in 0.5 ml of saline, 0.1 ml of 30% (w/v) TCA was added, and the suspension was mixed and was then centrifuged to separate the TCA-soluble and TCA pellet fractions. TCA-pellet fractions were removed for gamma counting. The percent of total cpm from PT and DT cells that was recovered in the TCA-pellet fraction is plotted *vs*. incubation time. Results are mean ± S.E. of measurements from four separate cell preparations. Statistical analyses: The following pairs ([Hg]/time) were significantly different ($P < .05$) from each other: For PT cells, 0.5/1 and 100/1, 0.5/10 and 100/10, 0.5/60 and 100/60, 1/1 and 100/1, 1/10 and 100/10, 1/60 and 100/60, 10/60 and 100/60. For DT cells, 0.5/1 and 100/1, 0.5/5 and 100/5, 0.5/10 and 100/10, 0.5/60 and 100/60, 1/1 and 100/1, 1/5 and 100/5, 1/10 and 100/10, 1/60 and 100/60, 10/1 and 100/1, 10/5 and 100/5, 10/10 and 100/10, 10/60 and 100/60. For PT cells *vs*. DT cells, none.

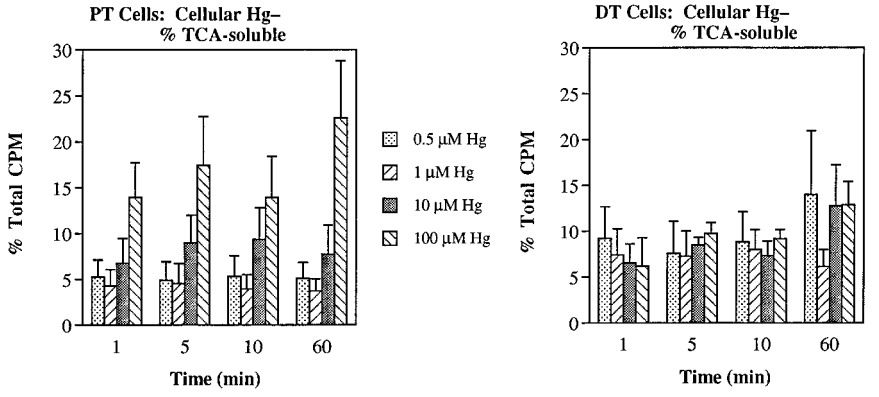

Fig. 3. Distribution of Hg: Cellular Hg-TCA supernatant. PT and DT cells from rat kidneys (1.2×10^6 cells/ml) were incubated with $^{203}HgCl_2$ and processed as described in the legend to figure 1. TCA-soluble and TCA pellet fractions were separated as described in the legend to figure 2. TCA-soluble fractions were removed for gamma counting. The percent of total cpm from PT and DT cells that was recovered in the TCA-soluble fraction is plotted versus incubation time. Results are mean ± S.E. of measurements from four separate cell preparations. Statistical analyses: The following pairs ([Hg]/time) were significantly different ($P < .05$) from each other: For PT cells, 0.5/1 and 100/1, 0.5/5 and 100/5, 0.5/10 and 100/10, 0.5/60 and 100/60, 1/1 and 100/1, 1/5 and 100/5, 1/10 and 100/10, 1/60 and 100/60, 10/10 and 100/10, 10/60 and 100/60, 100/1 and 100/60, 100/5 and 100/60. For DT cells, none. For PT cells *vs*. DT cells, 0.5/60, 100/60.

Influence of extracellular thiols on accumulation of Hg in renal PT and DT cells exposed to 0.1 μM Hg. The time course of cellular accumulation of Hg in PT and DT cells exposed to .1 μM Hg shows rapid accumulation of Hg in both cell types (fig. 4). No significant differences were detected between the two cell types at corresponding times. Within the first minute, total cellular Hg content reached ~50% of the approximate maximal level that was reached after 60 min of incubation.

Total amounts of Hg in extracellular media and cell pellets for cells incubated with 0.1 μM Hg alone ("buffer") or cells incubated with 0.1 μM Hg plus 0.4 μM of either BSA, DMPS, GSH or Cys are summarized in tables 1 and 2 for PT and DT cells, respectively. During 60 min of incubation with Hg alone, the amount of Hg in the supernatants gradually decreased over time to ~37% of the theoretical initial supernatant amount of 100 pmol. Cellular accumulation of Hg was most rapid during the first minute. Nearly 50% of the total accumulation of Hg that occurred over the entire 60-min incubation was observed during the first minute. For both PT and DT cells, coexposure to either Hg plus BSA or Hg plus DMPS resulted in the lowest amount of accumulation of Hg relative to exposure to Hg alone. On average, accumulation of Hg after exposure to Hg plus BSA or Hg plus DMPS was ~33% to 60% lower than that after exposure to Hg alone. Incubation of cells with Hg plus GSH also resulted in decreased Hg accumulation, but this coexposure resulted in smaller decreases in Hg accumulation than coexposure to either Hg plus BSA or Hg plus DMPS. Cellular contents of Hg after exposure to Hg plus GSH were 40% to 50% lower than those after exposure to Hg alone at the 1-min time point, ~30% lower after the 5-min time point, but were not significantly different at later time points. In contrast to coexposure to Hg with the other thiols, cell pellet contents of PT cells exposed to Hg plus Cys were modestly, but significantly, elevated compared with those detected after exposure to Hg alone, whereas those in DT cells exhibited no significant differences from values after exposure to Hg alone.

The relative effects of the four extracellular thiols on the disposition of Hg are shown graphically in figure 5. Exposure of PT and DT cells to Hg plus BSA or Hg plus DMPS exhibited the lowest amounts of cellular Hg accumulation com-

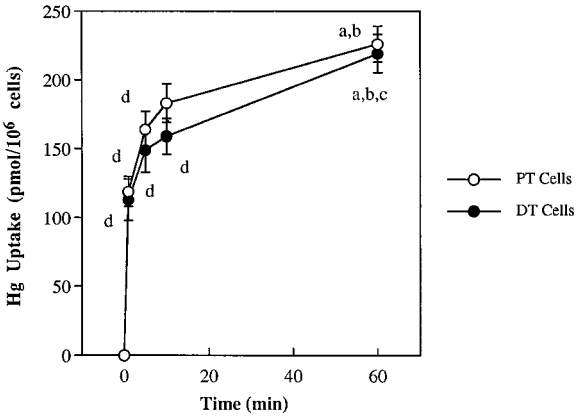

Fig. 4. Time course of renal PT and DT cellular accumulation of 0.1 μM Hg. Incubation mixtures (1.0 ml) contained 0.75 ml of appropriate $HgCl_2$ stock solution (0.05 μCi $^{203}HgCl_2$, cold $HgCl_2$ to give a final Hg concentration of 0.1 μM) and 0.25 ml of PT or DT cells (1.2×10^6 cells/ml) in 1.5-ml microcentrifuge tubes. Mixtures were incubated at 37°C with shaking in a Dubnoff water bath for 1, 5, 10 or 60 min. Hg accumulation was stopped by centrifugation for 2 min at 13,000 × g. Supernatants were transferred to 2.0-ml cryovials with a 1.0-ml Hamilton gastight syringe. The syringe was rinsed with 0.5 ml of saline, and the rinse was added to the supernatant (total volume, 1.5 ml). Radioactivity in supernatants and cell pellets was then determined in a gamma counter. Results are the mean ± S.E. of measurements from four or five separate cell preparations. Significantly different ($P < .05$) pairs: a, *vs.* 1-min time point in same cell type; b, *vs.* 5-min time point in same cell type; c, *vs.* 10-min time point in same cell type; d, *vs.* 60-min time point in same cell type; e, *vs.* DT cells at same time point; f, *vs.* PT cells at same time point.

pared with those detected after exposure to Hg alone. During exposure to Hg plus BSA or Hg plus DMPS in both cell types, Hg accumulation was 40% to 70% lower than that which was detected after exposure to Hg alone over the 60-min time course. By contrast, Hg accumulation in both PT and DT cells incubated with Hg plus GSH was only modestly lower (20–30%) than that in cells incubated with Hg alone, and this occurred only at the first two time points. Hg accumulation in PT cells exposed to Hg plus Cys was significantly higher than that in PT cells exposed to Hg alone at the 5- and 10-min time points. No significant differences in the cellular contents of Hg between DT cells exposed to Hg plus Cys and Hg alone were detected at any time point studied.

Influence of extracellular thiols on accumulation of Hg in renal PT and DT cells exposed to 1 μM Hg. The time course for cellular accumulation of 1 μM Hg was also rapid, with maximal levels essentially reached by 10 min (fig. 6). In contrast to the results obtained after exposure to 0.1 μM Hg, during exposure to 1 μM Hg, maximal content of Hg in PT cells was ~30% higher than that in DT cells.

Contents of Hg in extracellular supernatants in both PT cells (table 3) and DT cells (table 4) incubated with either Hg plus BSA, Hg plus DMPS, Hg plus GSH or Hg plus Cys were significantly higher than in cells incubated with Hg alone at all time points, indicating a marked reduction in cellular extraction of Hg in the presence of extracellular thiols. As with the case after exposure to 0.1 μM Hg, the smallest amount of Hg accumulation was found after coexposure to Hg plus BSA or Hg plus DMPS. In PT cells, Hg accumulation after coexposure to either Hg plus BSA or Hg plus DMPS was >80% lower than that after exposure to Hg alone. In DT cells, Hg accumulation after coexposure to Hg plus BSA or Hg plus DMPS was reduced by 85% or >90%, respectively, relative to that after exposure to Hg alone. Coexposure to Hg plus Cys resulted in the highest amounts of Hg accumulation of all the Hg plus thiol coexposures, whereas coexposure to Hg plus GSH resulted in intermediate accumulation values. Cellular accumulation of Hg after exposure to Hg plus Cys was greater in PT cells than in DT cells, which is consistent with the results with 0.1 μM Hg, in which coexposure with Hg plus Cys increased Hg accumulation relative to exposure to Hg alone in PT cells only. Cellular accumulation of Hg after coexposure to Hg plus GSH for 1 min was 82% and 75% lower in PT and DT cells, respectively, than that after exposure to Hg alone. After 60 min, however, Hg accumulation was only 57% and 71% lower in PT and DT cells, respectively, than that after exposure to Hg alone.

Direct comparison of effects of the four thiols on Hg accumulation, expressed as a percentage of Hg alone, in incubations with 1 μM Hg (fig. 7) showed clearly that coexposure with Hg plus DMPS produced the lowest amounts of Hg accumulation in both PT and DT cells: Cellular Hg contents

TABLE 1

Total extracellular and cellular Hg content in renal PT cells incubated with 0.1 μM Hg: Effect of extracellular thiols

Incubation mixtures (1.0 ml) contained 0.75 ml of appropriate $HgCl_2$ stock solution (0.05 μCi of $^{203}HgCl_2$, cold $HgCl_2$ to give a final Hg concentration of 0.1 μM and buffer or the indicated thiol compound at a final concentration of 0.4 μM) and 0.25 ml of PT cells (1.2×10^6 cells/ml) in 1.5-ml microcentrifuge tubes. Mixtures were incubated at 37°C with shaking in a Dubnoff water bath for 1, 5, 10 or 60 min. Hg accumulation was stopped by centrifugation for 2 min at 13,000 × *g*. Supernatants were transferred to 2.0-ml cryovials with a 1.0-ml Hamilton gastight syringe. The syringe was rinsed with 0.5 ml of saline, and the rinse was added to the supernatant (total volume, 1.5 ml). Radioactivity in supernatants and cell pellets was then determined in a gamma counter. Results are the mean ± S.E. of measurements from four or five separate cell preparations.

Incubation/time	Extracellular Hg	Cellular Hg
min	*pmol*	
Experiment 1		
Buffer/1	50.7 ± 9.5	31.1 ± 5.8
Buffer/5	52.5 ± 10.5	39.2 ± 8.0
Buffer/10	45.6 ± 9.6	43.5 ± 10.8
Buffer/60	36.8 ± 6.1	55.5 ± 11.2
BSA/1	69.6 ± 9.0	10.8 ± 4.7[a]
BSA/5	65.2 ± 6.5	11.6 ± 4.0[a]
BSA/10	64.6 ± 10.7	13.3 ± 3.6[a]
BSA/60	48.5 ± 7.1	28.2 ± 10.4[a]
Experiment 2		
Buffer/1	59.8 ± 15.2	23.5 ± 3.7
Buffer/5	56.0 ± 15.4	38.6 ± 5.2
Buffer/10	54.2 ± 13.4	51.9 ± 9.0
Buffer/60	37.6 ± 10.1	73.0 ± 10.9
DMPS/1	62.2 ± 4.8	13.9 ± 3.6[a]
DMPS/5	64.1 ± 13.3	20.1 ± 5.7[a]
DMPS/10	61.4 ± 6.6	25.3 ± 5.5[a]
DMPS/60	48.7 ± 10.3	42.5 ± 7.3[a]
Experiment 3		
Buffer/1	42.8 ± 6.3	50.4 ± 13.0
Buffer/5	41.1 ± 6.3	55.3 ± 10.7
Buffer/10	42.9 ± 6.0	55.0 ± 10.1
Buffer/60	37.6 ± 4.9	64.2 ± 7.9
GSH/1	61.7 ± 5.5[a]	28.2 ± 2.3[a]
GSH/5	56.8 ± 1.6[a]	40.4 ± 2.7[a]
GSH/10	50.5 ± 2.0	44.9 ± 3.4
GSH/60	44.5 ± 4.8	54.2 ± 4.6
Experiment 4		
Buffer/1	59.8 ± 5.7	35.2 ± 4.0
Buffer/5	52.9 ± 5.5	54.8 ± 6.4
Buffer/10	50.7 ± 5.3	62.5 ± 6.1
Buffer/60	37.0 ± 5.5	75.3 ± 4.5
Cys/1	60.7 ± 8.4	40.2 ± 3.7
Cys/5	50.6 ± 8.3	59.8 ± 5.9
Cys/10	47.4 ± 8.2	70.3 ± 6.0[a]
Cys/60	37.9 ± 7.2	80.6 ± 5.9

[a] Significantly different ($P < .05$) from corresponding value in paired samples incubated with Hg alone ("buffer").

TABLE 2
Total extracellular and cellular Hg content in renal DT cells incubated with 0.1 μM Hg: Effect of extracellular thiols

Hg accumulation in renal DT cells was measured as described in the legend to table 1. Results are the mean ± S.E. of measurements from four or five separate cell preparations.

Incubation/time	Extracellular Hg	Cellular Hg
min	*pmol*	
Experiment 1		
Buffer/1	66.3 ± 17.6	24.0 ± 10.3
Buffer/5	65.6 ± 11.6	36.8 ± 10.0
Buffer/10	57.9 ± 12.7	39.2 ± 9.3
Buffer/60	43.8 ± 11.0	51.9 ± 12.3
BSA/1	51.9 ± 10.8	12.6 ± 5.1[a]
BSA/5	70.7 ± 12.9	11.5 ± 5.4[a]
BSA/10	65.0 ± 5.6	11.1 ± 4.7[a]
BSA/60	57.1 ± 9.6	17.7 ± 1.8[a]
Experiment 2		
Buffer/1	64.0 ± 23.3	21.2 ± 2.2
Buffer/5	62.5 ± 24.2	32.6 ± 2.1
Buffer/10	63.4 ± 15.8	38.6 ± 5.5
Buffer/60	44.0 ± 8.6	67.8 ± 10.2
DMPS/1	74.6 ± 5.9	6.27 ± 1.55[a]
DMPS/5	70.3 ± 8.8	10.9 ± 1.9[a]
DMPS/10	74.8 ± 7.7	13.4 ± 1.1[a]
DMPS/60	51.0 ± 8.0	41.2 ± 5.7[a]
Experiment 3		
Buffer/1	44.6 ± 5.8	53.1 ± 11.9
Buffer/5	38.6 ± 6.4	58.8 ± 9.5
Buffer/10	39.5 ± 5.1	59.1 ± 8.2
Buffer/60	35.9 ± 3.5	70.8 ± 7.3
GSH/1	69.5 ± 3.8[a]	27.4 ± 2.5[a]
GSH/5	56.7 ± 2.0[a]	40.1 ± 1.7[a]
GSH/10	52.7 ± 1.1[a]	45.1 ± 2.9
GSH/60	35.4 ± 4.7[a]	61.8 ± 4.5
Experiment 4		
Buffer/1	70.7 ± 6.1	32.2 ± 6.4
Buffer/5	62.2 ± 5.8	47.0 ± 8.8
Buffer/10	61.1 ± 5.2	49.3 ± 6.6
Buffer/60	43.0 ± 4.8	70.4 ± 5.4
Cys/1	69.7 ± 8.2	29.4 ± 2.5
Cys/5	70.4 ± 8.7	39.9 ± 3.3
Cys/10	64.6 ± 7.9	49.7 ± 3.8
Cys/60	46.5 ± 7.4	68.9 ± 3.8

[a] Significantly different ($P < .05$) from corresponding value in paired samples incubated with Hg alone ("buffer").

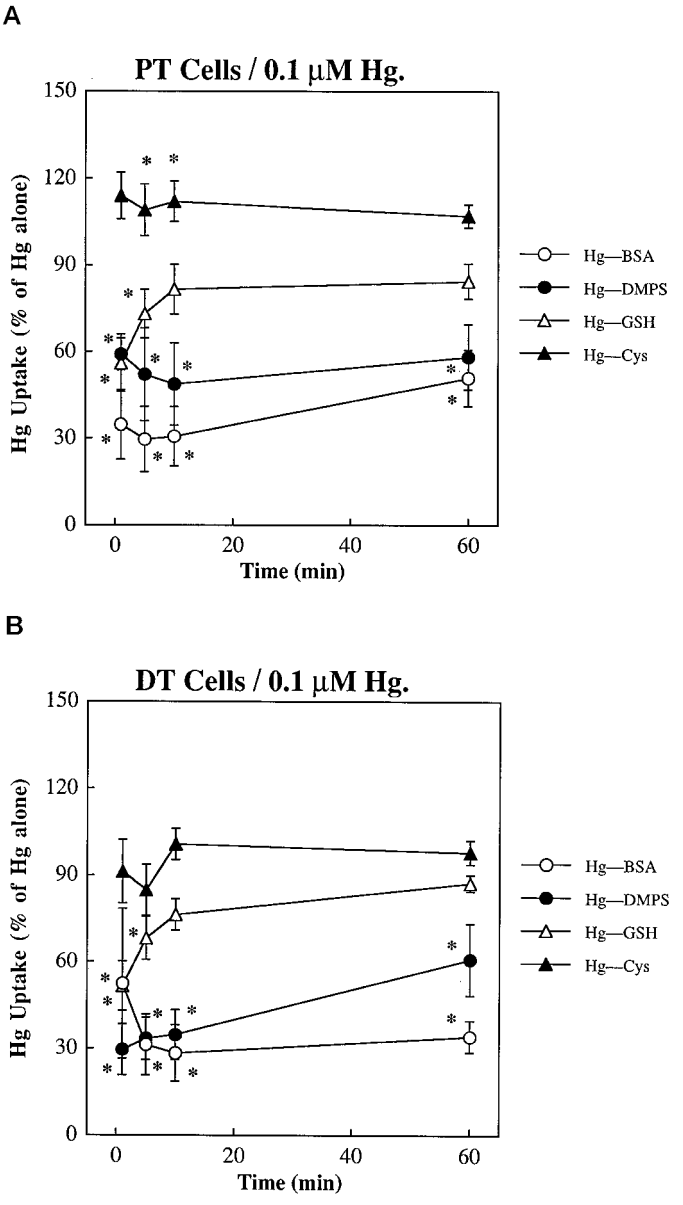

Fig. 5. Modulation of renal PT and DT cellular accumulation of 0.1 μM Hg by extracellular thiols. Cellular accumulation of 0.1 μM Hg in the presence of buffer or 0.4 μM thiol in PT cells (A) or DT cells (B) was measured as described in the legend to figure 4. Results are the mean ± S.E. of measurements from four or five separate cell preparations. *Significantly different ($P < .05$) from Hg added alone at same time point.

in the presence of Hg plus DMPS were <10% of those in the presence of Hg alone at all time points. At the 1 μM concentration of Hg, coexposure with 4 μM BSA or 4 μM GSH resulted in approximately equivalent amounts of cellular accumulation of Hg, with values ranging from 20% to 40% of those after exposure to Hg alone in PT cells and from 18% to 30% of those after exposure to Hg alone in DT cells. Coexposure with Cys resulted in the highest amounts of Hg accumulation of the four thiol coexposures, but this effect was greater in PT cells than in DT cells (maximal Hg accumulation was ~65% and 45% of Hg alone, respectively). An important difference in both cell types in the time course of Hg accumulation after exposure to Hg plus Cys or Hg plus GSH as compared with that after exposure to Hg plus BSA or Hg plus DMPS was that the percentage of decrease in Hg accumulation relative to that after exposure to Hg alone diminished with time of exposure to Hg plus Cys or Hg plus GSH but increased or remained stable with time of exposure to Hg plus BSA or Hg plus DMPS.

Influence of extracellular thiols on accumulation of Hg in renal PT and DT cells exposed to 5 μM Hg. The time courses for accumulation of Hg after exposure to 5 μM Hg were similarly rapid, reaching equilibrium values in both cell types within 10 min (fig. 8). In contrast to the results with exposure to 1 μM Hg, no significant differences in the cellular accumulation of Hg were observed between PT and DT cells after exposure to 5 μM Hg.

The amount of Hg in the extracellular supernatant was greater when cells were exposed to Hg plus BSA, Hg plus DMPS or Hg plus GSH compared with Hg alone at 5 μM Hg (tables 5 and 6). Furthermore, the fraction of Hg that was removed from the extracellular space was greatest with coexposure to one of the three thiols with 5 μM Hg than with either of the two lower concentrations of Hg that were tested. Amounts of Hg in the supernatant fractions of both PT and DT cells after 60-min exposures were >2-fold higher in cells exposed to Hg plus BSA than in those exposed to Hg alone, >4-fold higher in cells exposed to Hg plus DMPS and ~1.5-fold higher in cells exposed to Hg plus GSH. As with the

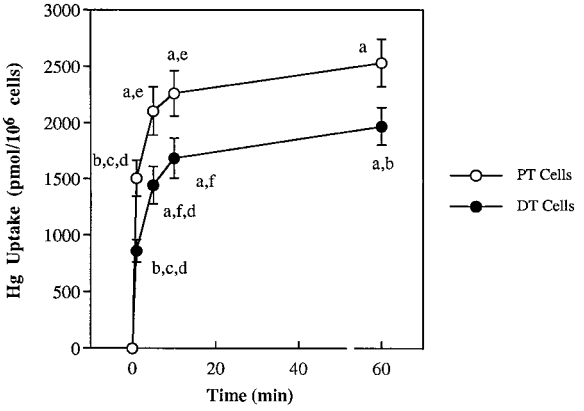

Fig. 6. Time course of renal PT and DT cellular accumulation of 1 μM Hg. Cellular accumulation of 1 μM Hg was measured as described in the legend to figure 4. Results are the mean ± S.E. of measurements from four or five separate cell preparations. Significantly different ($P < .05$) pairs: a, *vs*. 1-min time point in same cell type; b, *vs*. 5-min time point in same cell type; c, *vs*. 10-min time point in same cell type; d, *vs*. 60-min time point in same cell type; e, *vs*. DT cells at same time point; f, *vs*. PT cells at same time point.

TABLE 3

Total extracellular and cellular Hg content in renal PT cells incubated with 1 μM Hg: Effect of extracellular thiols

Hg accumulation in renal PT cells was measured as described in the legend to table 1 except that final Hg and thiol concentrations were 1 and 4 μM, respectively. Results are the mean ± S.E. of measurements from four or five separate cell preparations.

Incubation/time	Extracellular Hg	Cellular Hg
min	*pmol*	
Experiment 1		
Buffer/1	525 ± 94	168 ± 72
Buffer/5	495 ± 104	233 ± 96
Buffer/10	459 ± 112	290 ± 117
Buffer/60	414 ± 107	352 ± 119
BSA/1	776 ± 70[a]	35.8 ± 7.9[a]
BSA/5	746 ± 90[a]	40.1 ± 7.4[a]
BSA/10	699 ± 93[a]	44.7 ± 10.7[a]
BSA/60	680 ± 109[a]	68.0 ± 16.5[a]
Experiment 2		
Buffer/1	393 ± 102	408 ± 103
Buffer/5	301 ± 56	614 ± 122
Buffer/10	282 ± 34	691 ± 105
Buffer/60	240 ± 28	744 ± 78
DMPS/1	833 ± 58[a]	35.9 ± 8.4[a]
DMPS/5	806 ± 14[a]	40.5 ± 12.0[a]
DMPS/10	740 ± 42[a]	54.0 ± 22.8[a]
DMPS/60	721 ± 48[a]	69.7 ± 18.8[a]
Experiment 3		
Buffer/1	519 ± 27	376 ± 70
Buffer/5	418 ± 15	531 ± 45
Buffer/10	405 ± 13	566 ± 42
Buffer/60	379 ± 9	622 ± 42
GSH/1	703 ± 67[a]	69.0 ± 5.9[a]
GSH/5	686 ± 75[a]	107 ± 15[a]
GSH/10	705 ± 47[a]	125 ± 21[a]
GSH/60	602 ± 72[a]	269 ± 61[a]
Experiment 4		
Buffer/1	556 ± 38	602 ± 61
Buffer/5	421 ± 37	804 ± 89
Buffer/10	412 ± 42	837 ± 77
Buffer/60	350 ± 47	943 ± 85
Cys/1	810 ± 80[a]	189 ± 37[a]
Cys/5	768 ± 71[a]	277 ± 43[a]
Cys/10	718 ± 51[a]	392 ± 78[a]
Cys/60	627 ± 63[a]	616 ± 87[a]

[a] Significantly different ($P < .05$) from corresponding value in paired samples incubated with Hg alone ("buffer").

TABLE 4

Total extracellular and cellular Hg content in renal DT cells incubated with 1 μM Hg: Effect of extracellular thiols

Hg accumulation was measured in renal DT cells as described in the legend to table 1 except that final Hg and thiol concentrations were 1 and 4 μM, respectively. Results are the mean ± S.E. of measurements from four or five separate cell preparations.

Incubation/time	Extracellular Hg	Cellular Hg
min	*pmol*	
Experiment 1		
Buffer/1	564 ± 71	125 ± 28
Buffer/5	411 ± 73	228 ± 63
Buffer/10	355 ± 67	264 ± 94
Buffer/60	375 ± 70	349 ± 79
BSA/1	782 ± 87[a]	35.1 ± 6.4[a]
BSA/5	819 ± 76[a]	35.0 ± 10.5[a]
BSA/10	835 ± 73[a]	50.1 ± 22.9[a]
BSA/60	751 ± 80[a]	51.4 ± 9.5[a]
Experiment 2		
Buffer/1	385 ± 74	228 ± 52
Buffer/5	377 ± 64	450 ± 22
Buffer/10	327 ± 26	498 ± 119
Buffer/60	355 ± 12	588 ± 105
DMPS/1	811 ± 9[a]	21.2 ± 4.5[a]
DMPS/5	811 ± 15[a]	27.1 ± 0.9[a]
DMPS/10	784 ± 46[a]	26.2 ± 5.5[a]
DMPS/60	851 ± 49[a]	40.2 ± 4.2[a]
Experiment 3		
Buffer/1	536 ± 51	223 ± 39
Buffer/5	472 ± 41	351 ± 19
Buffer/10	470 ± 36	425 ± 33
Buffer/60	469 ± 21	503 ± 22
GSH/1	674 ± 78[a]	54.5 ± 4.1[a]
GSH/5	728 ± 59[a]	63.0 ± 9.1[a]
GSH/10	746 ± 42[a]	73.0 ± 11.7[a]
GSH/60	661 ± 61[a]	144 ± 21[a]
Experiment 4		
Buffer/1	551 ± 47	340 ± 47
Buffer/5	486 ± 51	554 ± 83
Buffer/10	499 ± 48	627 ± 79
Buffer/60	453 ± 38	727 ± 70
Cys/1	759 ± 82[a]	105 ± 21[a]
Cys/5	733 ± 81[a]	149 ± 31[a]
Cys/10	709 ± 64[a]	216 ± 47[a]
Cys/60	672 ± 64[a]	362 ± 73[a]

[a] Significantly different ($P < .05$) from corresponding value in paired samples incubated with Hg alone ("buffer").

lower concentrations of Hg, Hg accumulation after exposure to 5 μM Hg was lowest with coexposure with BSA or DMPS, whereas amounts of Hg in cells incubated with Hg plus GSH were significantly higher than in those incubated with either Hg plus BSA or Hg plus DMPS.

Direct comparison of Hg accumulation in cells exposed to Hg alone and those exposed to Hg plus BSA, Hg plus DMPS or Hg plus GSH shows clearly that the percent decrease of Hg accumulation was much greater at 5 μM Hg than at either of the two lower concentrations studied (fig. 9). Another difference in the time course with exposure to 5 μM Hg compared with exposure to 1 μM or 0.1 μM Hg was that there was little if any change in the percent decrease of Hg accumulation during the course of the 60-min incubation in cells exposed to Hg plus GSH. Rather, the time courses for Hg accumulation in each of the Hg plus thiol coexposures exhibited the same pattern in both PT and DT cells.

Discussion

The high affinity of Hg for sulfhydryl groups plays a critical role in its disposition and mechanism of toxicity (Zalups and Lash, 1994). Hence, alteration of either intracellular or ex-

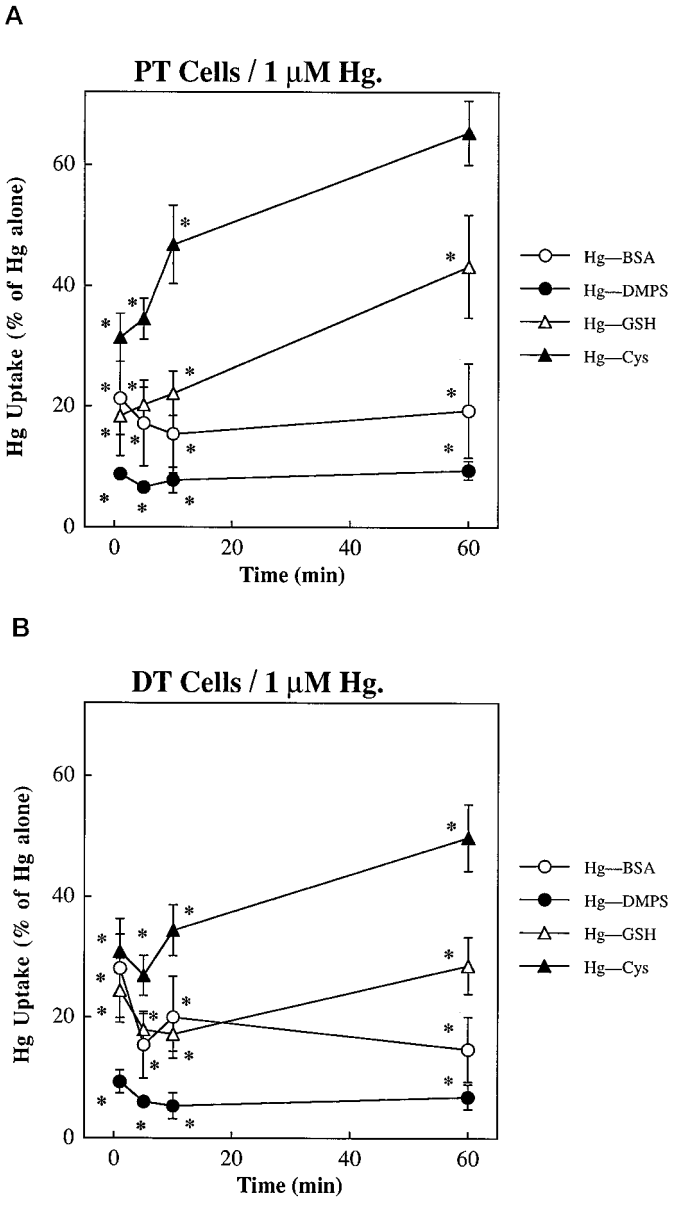

Fig. 7. Modulation of renal PT and DT cellular accumulation of 1 μM Hg by extracellular thiols. Cellular accumulation of 1 μM Hg in the presence of buffer or 4 μM thiol in PT cells (A) or DT cells (B) was measured as described in the legend to figure 4. Results are the mean ± S.E. of measurements from four or five separate cell preparations. *Significantly different ($P < .05$) from Hg added alone at same time point.

tracellular thiol status should affect the renal uptake and cellular accumulation of Hg. Indeed, several studies have demonstrated that either alteration of intracellular thiol content or coadministration of thiols with Hg can have profound effects on the uptake and toxicity of Hg in the target organ, the kidney. The relationship between intrarenal Hg content and the severity of Hg-induced nephropathy, however, is a difficult one to define mechanistically. On the one hand, a dose response exists for Hg, although the curve is extremely steep, with almost an "all-or-none" response often being observed. On the other hand, although higher doses of Hg do lead to higher intrarenal contents of Hg, as doses are increased from low levels, there is little correlation between uptake and renal cellular accumulation of Hg once intoxication has occurred (Zalups *et al*., 1988). An example of this is

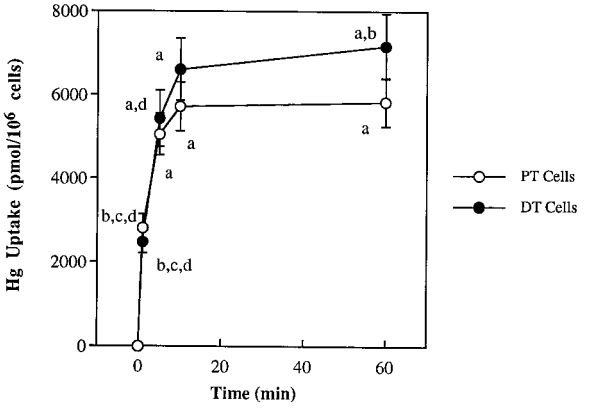

Fig. 8. Time course of renal PT and DT cellular accumulation of 5 μM Hg. Cellular accumulation of 5 μM Hg was measured as described in the legend to figure 4. Results are the mean ± S.E. of measurements from four or five separate cell preparations. Significantly different ($P < .05$) pairs: a, *vs*. 1-min time point in same cell type; b, *vs*. 5-min time point in same cell type; c, *vs*. 10-min time point in same cell type; d, *vs*. 60-min time point in same cell type; e, *vs*. DT cells at same time point; f, *vs*. PT cells at same time point.

TABLE 5

Total extracellular and cellular Hg content in renal PT cells incubated with 5 μM Hg: Effect of extracellular thiols

Hg accumulation in renal PT cells was measured as described in the legend to table 1 except that final Hg and thiol concentrations were 5 μM and 20 μM, respectively. Results are the mean ± S.E. of measurements from four or five separate cell preparations.

Incubation/time	Extracellular Hg	Cellular Hg
min	*pmol*	
Experiment 1		
Buffer/1	2565 ± 611	666 ± 138
Buffer/5	2121 ± 465	1174 ± 198
Buffer/10	1930 ± 413	1312 ± 238
Buffer/60	1658 ± 337	1454 ± 276
BSA/1	3616 ± 313	92.8 ± 18.5[a]
BSA/5	3494 ± 308[a]	139 ± 13[a]
BSA/10	3392 ± 472[a]	162 ± 21[a]
BSA/60	3682 ± 236[a]	185 ± 25[a]
Experiment 2		
Buffer/1	2505 ± 648	822 ± 301
Buffer/5	988 ± 142	1328 ± 80
Buffer/10	1131 ± 333	1529 ± 151
Buffer/60	1110 ± 275	1427 ± 78
DMPS/1	5056 ± 195[a]	130 ± 17[a]
DMPS/5	3645 ± 792[a]	112 ± 35[a]
DMPS/10	3765 ± 785[a]	117 ± 33[a]
DMPS/60	4009 ± 617[a]	178 ± 70[a]
Experiment 3		
Buffer/1	3282 ± 430	1035 ± 158
Buffer/5	2602 ± 371	1785 ± 148
Buffer/10	2306 ± 307	2045 ± 208
Buffer/60	2125 ± 353	2122 ± 266
GSH/1	3430 ± 362	205 ± 30[a]
GSH/5	3005 ± 567	244 ± 16[a]
GSH/10	3455 ± 406[a]	280 ± 33[a]
GSH/60	3284 ± 546[a]	391 ± 49[a]

[a] Significantly different ($P < .05$) from corresponding value in paired samples incubated with Hg alone ("buffer").

seen in the present study, where exposure of renal PT and DT cells to concentrations of Hg of >10 μM resulted in decreases in the percentage of Hg recovered in the cell pellets and increases in the percent of Hg recovered in the acid-soluble fraction of cells (compare figs. 2 and 3). This agrees with the concentration dependence of Hg-induced renal cellular injury in PT cells that we observed previously (Lash and Zalups, 1992), where exposure of cells to Hg concentrations above 10 μM produced marked increases in cell death.

TABLE 6

Total extracellular and cellular Hg content in renal DT cells incubated with 5 μM Hg: Effect of extracellular thiols

Hg accumulation in renal DT cells was measured as described in the legend to table 1 except that final Hg and thiol concentrations were 5 and 20 μM, respectively. Results are the mean ± S.E. of measurements from four or five separate cell preparations.

Incubation/time	Extracellular Hg	Cellular Hg
min	*pmol*	
Experiment 1		
Buffer/1	2605 ± 817	904 ± 276
Buffer/5	2121 ± 551	1046 ± 212
Buffer/10	1831 ± 355	1505 ± 269
Buffer/60	1603 ± 242	1609 ± 359
BSA/1	3858 ± 134	121 ± 12[a]
BSA/5	3509 ± 404[a]	110 ± 16[a]
BSA/10	3618 ± 349[a]	119 ± 18[a]
BSA/60	3568 ± 252[a]	158 ± 22[a]
Experiment 2		
Buffer/1	1723 ± 402	616 ± 114
Buffer/5	1254 ± 225	2109 ± 518
Buffer/10	1233 ± 118	2287 ± 586
Buffer/60	870 ± 77	2335 ± 605
DMPS/1	3780 ± 470[a]	84.0 ± 28.9[a]
DMPS/5	4343 ± 393[a]	108 ± 23[a]
DMPS/10	3723 ± 695[a]	86.3 ± 28.1[a]
DMPS/60	4327 ± 548[a]	111 ± 28[a]
Experiment 3		
Buffer/1	3512 ± 595	633 ± 90
Buffer/5	2910 ± 242	1269 ± 404
Buffer/10	2378 ± 336	1996 ± 238
Buffer/60	2061 ± 164	2256 ± 244
GSH/1	4150 ± 99	185 ± 25[a]
GSH/5	4038 ± 181[a]	185 ± 26[a]
GSH/10	3863 ± 350[a]	204 ± 24[a]
GSH/60	3326 ± 453[a]	341 ± 44[a]

[a] Significantly different ($P < .05$) from corresponding value in paired samples incubated with Hg alone ("buffer").

Rapid accumulation of Hg in renal PT cells is expected because these cells are the primary *in vivo* sites of Hg accumulation (Zalups, 1991a, 1991b; Zalups and Lash, 1994). In a study of Hg accumulation in microdissected nephron segments from rabbits given a subtoxic dose of Hg, maximal accumulation of Hg was found in the proximal tubular segments and no significant accumulation of Hg was detected in nephron segments distal to the proximal tubule (Zalups and Barfuss, 1990). Similar findings were obtained from nephron segments isolated from rats treated with a subtoxic dose of Hg (Zalups, 1991a). Based on these results, the isolated renal DT cells might not be expected to accumulate significant amounts of Hg. However, depending on the concentration of Hg administered, the accumulation of Hg in DT cells was similar to or only modestly lower than values in PT cells (compare figs. 2–4, 6 and 8). Differences in the nature of the *in vitro* model systems used may contribute to these results. With isolated PT and DT cells, the normal tubular polarity that separates luminal from basolateral plasma membranes is lost and all surfaces have equal access to compounds in the extracellular medium. With isolated nephron segments, in contrast, intact polarity is maintained. Another possible explanation is that the observed cellular accumulation of Hg in the DT cell preparation may reflect that which is occurring in contaminating PT cells. However, this explanation is not likely to account for more than a small portion of the observed accumulation of Hg since, based on enzymatic and functional markers, the maximal amount of contamination of the DT cell preparation with PT cells is only 5% to 10% (Lash and Tokarz, 1989). Hence, we conclude that renal cells from

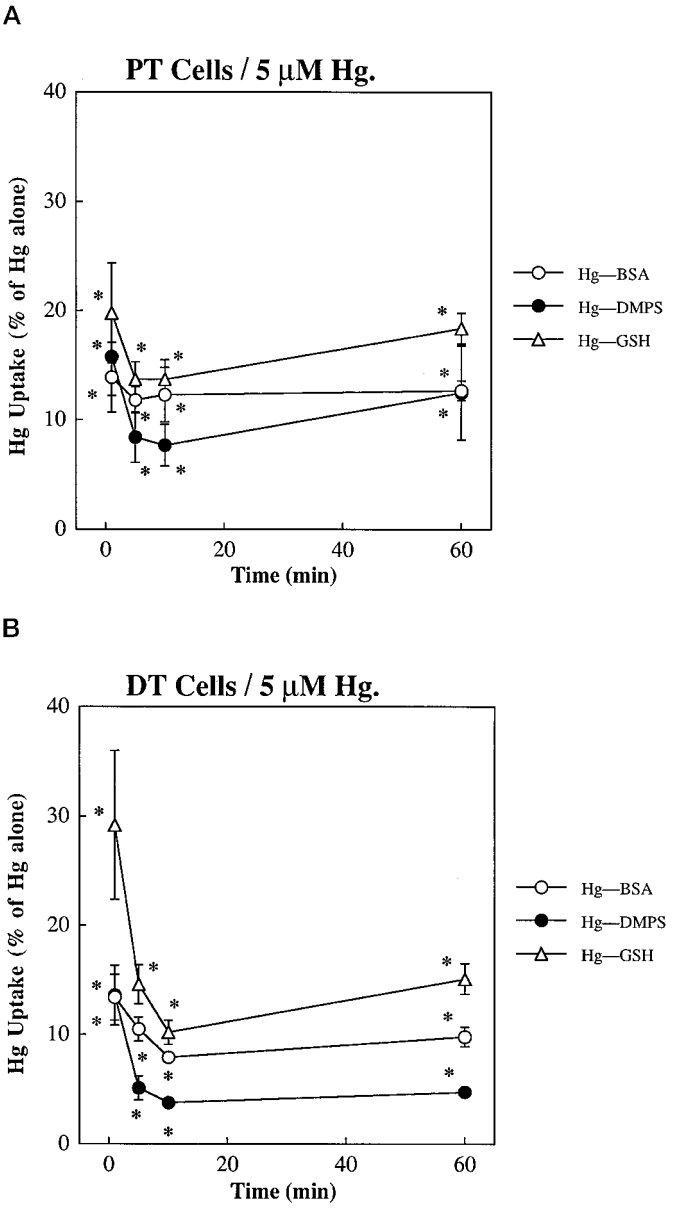

Fig. 9. Modulation of renal PT and DT cellular accumulation of 5 μM Hg by extracellular thiols. Cellular accumulation of 5 μM Hg in the presence of buffer or 20 μM thiol in PT cells (A) or DT cells (B) was measured as described in the legend to figure 4. Results are the mean ± S.E. of measurements from four or five separate cell preparations. *Significantly different ($P < .05$) from Hg added alone at same time point.

the DT region have the capacity to accumulate Hg but that *in vivo*, renal blood flow, the high affinity of Hg transport and binding in the PT region, and other factors alter the delivery of Hg so that minimal accumulation of Hg is observed in the DT region. This is the first report describing accumulation of Hg in a cell type derived from the distal nephron.

Although the general patterns of accumulation of Hg were the same in the two cell populations, some differences were observed. Concentration-dependent increases in recovery of Hg in the TCA-soluble and TCA-pellet fractions were observed in PT cells but not in DT cells. Comparison of the subcellular distribution patterns with the pattern of Hg-induced cellular injury in isolated PT cells (Lash and Zalups, 1992) suggests that the amount of Hg in the TCA-soluble

fraction appears to correlate with the degree of Hg-induced renal cellular injury.

The rapid accumulation of Hg in renal cells may be due in part to the decrease in the extracellular concentrations of Hg during the incubations. Although the ideal conditions would be to have constant extracellular concentrations of Hg over the entire time course of incubations, isolated PT fragments rapidly extract Hg, leading to the observed decreases in extracellular concentrations of Hg (see discussion in Zalups *et al*., 1993). It is difficult under the current experimental conditions of media volume and cell concentration to achieve these ideal conditions. Hence, there is a significant decrease in extracellular concentrations of Hg over time and as this occurs, the kinetics of Hg transport and/or binding change. *In vivo*, one also has a similar situation because after a given dose of Hg, there is a finite amount of Hg in plasma that is avidly extracted by the kidneys, leading to a situation where the concentration of Hg being presented to the kidneys decreases, thereby altering elimination kinetics.

The results from this study confirm previous findings and support the hypothesis that extracellular thiols modulate cellular accumulation of Hg. BSA and DMPS, regardless of concentration, decreased Hg accumulation in both cell populations. The extent of the decrease, however, increased with increasing incubation time and concentration of Hg, despite the constancy of the thiol:Hg ratio. Although DMPS itself is avidly transported into renal PT cells (Klotzbach and Diamond, 1988), the present results showing minimal accumulation of an Hg-DMPS complex in PT and DT cells are consistent with the *in vitro* ability of DMPS to prevent uptake and binding of Hg in renal brush-border membrane vesicles (Zalups and Lash, 1997) and to be an extremely effective protective agent in renal PT cells against Hg-induced renal cellular injury (Lash and Zalups, 1992). DMPS is also a highly effective protective agent *in vivo* against Hg-induced nephropathy (Zalups *et al*., 1991a). The mode of *in vivo* protection by DMPS presumably involves extraction of cellular Hg by intracellular chelation, whereas under the conditions of the present study where DMPS was added simultaneously with Hg, the decrease in cellular accumulation of Hg is due to prevention or inhibition of Hg transport and/or binding in the cell. In a previous study (Lash and Zalups, 1992), in contrast, isolated PT cells were preloaded with DMPS and were completely protected from Hg-induced cellular injury, suggesting that intracellular chelation was the mechanism of protection.

The similarity in the ability of coexposure of renal cells with Hg plus BSA or Hg plus DMPS to decrease Hg accumulation raises an important question about the role of serum albumin as a physiological ligand for Hg and the ability of Hg that is bound to albumin to be extracted by the kidneys. A major difference exists, however, between conditions *in vivo* and those with the *in vitro* exposures conducted in the present study. In the *in vivo* state, other thiols, including GSH and Cys, are present when the Hg-albumin complex reaches the kidneys. Accordingly, exchange between albumin and these low-molecular-weight thiols can occur, thereby facilitating renal cellular uptake and accumulation of Hg. In the present study, in contrast, cells were exposed to Hg plus BSA in the absence of other competing thiols, so additional ligands to which Hg can bind were not present. With these differences between *in vivo* and *in vitro* exposure conditions in mind, as well as the known ability of Hg bound to thiols to exchange with other ligands (Oram *et al*., 1996; Rabenstein, 1989), the hypothesis that complexes of Hg with albumin can function to deliver Hg to the target organ remains viable.

In contrast to the similarities in effects of BSA and DMPS on Hg accumulation, coexposure with a 4-fold molar excess of Cys exhibited different effects at 0.1 and 1 μM Hg. Coexposure of PT cells, but not DT cells, to 0.1 μM Hg and 0.4 μM Cys significantly enhanced Hg accumulation relative to incubations with Hg alone. Coexposure of both PT and DT cells to 1 μM Hg and 4 μM Cys, in contrast, significantly decreased Hg accumulation relative to incubations with Hg alone at early incubation times, and the extent of the decrease diminished with increasing time. The extent of the decrease in Hg accumulation with coexposure to Cys, however, was significantly less than that seen with coexposure to either BSA or DMPS. Hence, the present results are consistent with an Hg-Cys complex being transported into renal PT cells. Furthermore, these studies agree with a recent study (Zalups and Lash, 1997) that showed that coincubation of brush-border membrane vesicles from rat renal cortex with Hg and Cys markedly enhanced uptake and binding of Hg compared with membrane vesicles incubated with Hg alone.

At both 0.1 and 1 μM Hg and in both PT and DT cells, coexposure with 0.4 and 4 μM GSH, respectively, significantly decreased Hg accumulation relative to incubations with Hg alone, and the extent of the decrease diminished with incubation time. However, the accumulation of Hg in exposures to Hg-GSH was significantly higher than that in exposures to Hg-BSA or Hg-DMPS. In contrast, at 5 μM Hg in both PT and DT cells, coexposure with 20 μM GSH decreased Hg accumulation relative to incubations with Hg alone to a similar extent as did coexposure with 20 μM BSA or 20 μM DMPS.

It is clear that BSA and DMPS are extremely effective inhibitors of Hg accumulation in renal PT and DT cells, which corresponds with their ability to provide virtually complete protection of renal cells from Hg-induced injury (Lash and Zalups, 1992; Zalups, 1993b; Zalups *et al*., 1991a, 1991b, 1993). The results with Cys are consistent with an Hg-Cys complex functioning as a transport form of Hg. However, due to the apparent transport and/or binding kinetics, the Hg-Cys complex is transported more efficiently at low concentrations of Hg (*i.e*., <1 μM Hg) than at higher concentrations of Hg. Because renal PT cells have such excessively high GGT activity, it is likely that degradation of GSH or an Hg-GSH complex, producing, in combination with dipeptidase activity, Cys or an Hg-Cys complex, respectively, can occur (Visarius *et al*., 1996). Although it cannot be excluded that an Hg-GSH complex is a transport form of Hg, it is likely that at least some of the Hg-GSH complex is degraded to the corresponding Hg-Cys complex and that the latter complex is the transport form. Clarkson (1993) has previously suggested that thiol conjugates of Hg act like molecular mimics of other thiol conjugates and are thus transported and metabolized by analogous pathways. Transport of GSH and Cys conjugates of organic compounds such as trichloroethylene into renal PT cells occurs by both Na^+-dependent pathways and the organic anion transporter (Lash and Jones, 1985b; Lash and Anders, 1989). Conjugates of Hg with GSH or Cys may mimic such conjugates and may be transported into PT cells by the same pathways.

Another factor that should be taken into account is poten-

tial competition for transport between the Hg-thiol conjugates and the corresponding disulfides that may form from the thiols in solution due to autoxidation. There is evidence that the dibasic amino acid carrier, which transports cystine, may play a role in Hg-Cys transport across the renal brush-border membrane (R.K. Zalups and D.W. Barfuss, unpublished observations). This carrier, however, is a low-affinity transporter. If all the unbound Cys was oxidized to cystine, then the maximal concentration of cystine would be the same as that of Hg-Cys, which suggests that competition would be minimal. Nevertheless, the values for Hg accumulation with Hg-Cys may be underestimates because of this potential competition. Similarly, GSSG uptake across renal plasma membranes occurs by a low-affinity, low-capacity process (Lash and Jones, 1984), suggesting that competition between GSSG and Hg-GSH for transport would also be minimal.

It is critical to note that it is very unlikely that renal epithelial cells will be exposed *in vivo* to free Hg ions. Rather, Hg will be bound to a ligand (presumably albumin, GSH or Cys). Hence, the most relevant comparisons of Hg accumulation values in the present study are those among coexposures of renal cells to the various thiols plus Hg. Considered in this light, then, the higher amounts of cellular accumulation of Hg with exposures to Hg plus GSH or Hg plus Cys compared with Hg plus BSA or Hg plus DMPS take on added significance and provide stronger support for the hypothesis that complexes of Hg with GSH or Cys are physiological transport forms of Hg in renal epithelial cells.

Based on data from *in vivo* studies using ureteral ligation (Zalups and Minor, 1995) and inhibition of renal GGT activity by acivicin pretreatment (de Ceaurriz *et al*., 1994; Tanaka *et al*., 1990; Tanaka-Kagawa *et al*., 1993; Zalups, 1995) and *in vitro* studies using renal plasma membrane vesicles (Zalups and Lash, 1997), transport of the putative Hg-Cys or Hg-GSH complex can be localized to the brush-border membranes of PT cells. Additionally, there is strong evidence for a role for the renal organic anion transporter in the basolateral uptake of Hg (Zalups, 1995; Zalups and Barfuss, 1995c; Zalups and Minor, 1995). Hence, there are clearly two mechanisms, one luminal and one basolateral, for the renal cellular uptake and accumulation of Hg.

In conclusion, these studies have demonstrated that Hg is accumulated by both isolated PT and DT cells and is differentially distributed into soluble and protein-bound fractions. The hypothesis that extracellular thiols significantly modulate cellular accumulation of Hg is supported, confirming and extending findings with other *in vitro* models. Furthermore, these studies show that Cys and/or GSH are important physiological ligands for Hg that are involved in their transport into renal cells.

References

Baggett JMcC 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.

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

Bohets HH, Van Thielen MN, Van Der Biest I, Van Landeghem GF, D'Haese PC, Nouwen EJ, De Broe ME and Dierlickx PJ (1995) Cytotoxicity of mercury compounds in LLC-PK_1, MDCK and human proximal tubular cells. *Kidney Int* **47:** 395–403.

Burton CA, Hatlelid K, Divine K, Carter DE, Fernando Q, Brendel K and Gandolfi AJ (1995) Glutathione effects on toxicity and uptake of mercuric chloride and sodium arsenite in rabbit renal cortical slices. *Environ Health Perspec* **103**(Suppl. 1)**:**81–84.

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.

Clarkson TW (1993) Molecular and ionic mimicry of toxic metals. *Annu Rev Pharmacol Toxicol* **32:**545–571.

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.

Foulkes EC (1974) Excretion and retention of cadmium, zinc, and mercury by rabbit kidney. *Am J Physiol* **227:**1356–1360.

Friedman HL (1957) Relationship between chemical structure and biological activity in mercurial compounds. *Ann NY Acad Sci* **65:**461–470.

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.

Houser MT, Milner LS, Kolbeck PC, Wei SH and Stohs SJ (1992) Glutathione monoethyl ester moderates mercuric chloride-induced acute renal failure. *Nephron* **61:**449–455.

Johnson DR (1982) Role of renal cortical sulfhydryl groups in development of mercury-induced renal toxicity. *J Toxicol Environ Health* **9:**119–126.

Jones DP, Sundby G-B, Ormstad K and Orrenius S (1979) Use of isolated kidney cells for study of drug metabolism. *Biochem Pharmacol* **28:**929–935.

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.

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 (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 cyst(e)ine 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 (1997) Role of thiols in uptake and intracellular distribution of inorganic mercury in rat renal proximal tubular and distal tubular cells. *Fundam Appl Toxicol* **36** (Suppl.)**:**184.

Lash LH and Tokarz JJ (1989) Isolation of two distinct populations of cells from rat kidney cortex and their use in chemically-induced toxicity. *Anal Biochem* **182:**271–279.

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.

Lau S-J and Sarkar B (1979) Inorganic mercury(II)-binding components in normal human blood serum. *J Toxicol Environ Health* **5:**907–916.

Oram PD, Fang X, Fernando Q, Letkeman P and Letkemen D (1996) The formation constants of mercury (II)–glutathione complexes. *Chem Res Toxicol* **9:**709–712.

Rabenstein DL (1989) Metal complexes of glutathione and their biological significance, *In* Glutathione: Chemical, Biochemical and Medical Aspects, ed. by Dolphin D, Auramovic O and Poulson R, pp. 147–186, Wiley, New York.

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-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.

Visarius TM, Putt DA, Schare JM, Pegouske DM and Lash LH (1996) Pathways of glutathione metabolism and transport in isolated proximal tubular cells from rat kidney. *Biochem Pharmacol* **52:**259–272.

Zalups RK (1991a) 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 (1991b) 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 (1993a) 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 (1993b) Early aspects of the intrarenal distribution of mercury after the intravenous administration of mercuric chloride. *Toxicology* **79:**215–228.

Zalups RK (1995) Organic anion transport and action of γ-glutamyl transpeptidase in kidney linked mechanistically to renal tubular uptake of inorganic mercury. *Toxicol Appl Pharmacol* **132:**289–298.

Zalups RK and Barfuss D (1990) Accumulation of inorganic mercury along the renal proximal tubule of the rabbit. *Toxicol Appl Pharmacol* **106:**245–253.

Zalups RK and Barfuss DW (1995a) 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 (1995b) Accumulation and handling of inorganic mercury in the kidney after coadministration with glutathione. *J Toxicol Environ Health* **44:**385–399.

Zalups RK and Barfuss DW (1995c) 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 (1996) Nephrotoxicity of inorganic mercury coadministered with L-cysteine. *Toxicology* **109:**15–29.

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*, ed. by Clarkson TW, Friberg L, Nordberg GF and Sager PR, pp. 531–545, Plenum Publishing, New York.

Zalups RK and Diamond GL (1987) Mercuric chloride-induced nephrotoxicity in the

rat after unilateral nephrectomy and compensatory renal growth. *Virchows Arch B* **53:**336–346.
Zalups RK, Gelein RM and Cernichiari E (1991a) DMPS as a rescue agent for the nephropathy induced by mercuric chloride. *J Pharmacol Exp Ther* **256:**1–10.
Zalups RK, Knutson KL and Schnellmann RG (1993) *In vitro* analysis of the accumulation and toxicity of inorganic mercury in segments of the proximal tubule isolated from 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) Recent advances in understanding the renal transport and toxicity of mercury. *J Toxicol Environ Health* **42:**1–44.
Zalups RK and Lash LH (1997) Binding of inorganic mercury in renal brush-border and basolateral membrane-vesicles: Implication of a cysteine conjugate of mercury in the transport involved in the luminal uptake of inorganic mercury in the kidney. *Biochem Pharmacol* **53:**1889–1900.
Zalups RK, Klotzbach JM and Diamond GL (1987) Enhanced accumulation of injected inorganic mercury in renal outer medulla after unilateral nephrectomy. *Toxicol Appl Pharmacol* **89:**226–236.
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, Robinson MK and Barfuss DW (1991b) Factors affecting inorganic mercury transport and toxicity in the isolated perfused proximal tubule. *J Am Soc Nephrol* **2:**866–878.

Send reprint requests to: Lawrence H. Lash, Ph.D., Department of Pharmacology, Wayne State University, School of Medicine, 540 East Canfield Avenue, Detroit, MI 48201. E-mail: l.h.lash@wayne.edu