Participation of Mercuric Conjugates of Cysteine, Homocysteine, and N-Acetylcysteine in Mechanisms Involved in the Renal Tubular Uptake of Inorganic Mercury

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Abstract. Mechanisms involved in the renal uptake of inorganic mercury were studied in rats administered a nontoxic 0.5 μmol/kg intravenous dose of inorganic mercury with or without 2.0 μmol/kg cysteine, homocysteine, or N-acetylcysteine. The renal disposition of mercury was studied 1 h after treatment in normal rats and rats that had undergone bilateral ureteral ligation. In addition, the disposition of mercury (including the urinary and fecal excretion of mercury) was evaluated 24 h after treatment. In normal rats, coadministering inorganic mercury plus cysteine or homocysteine caused a significant increase in the renal uptake of mercury 1 h after treatment. The enhanced renal uptake of mercury was due to increased uptake of mercury in the renal outer stripe of the outer medulla and/or renal cortex. Ureteral ligation caused reductions in the renal uptake of mercury in all groups except for the one treated with inorganic mercury plus N-acetylcysteine. Thus, it appears that virtually all of the mercury taken up by the kidneys of the normal rats treated with inorganic mercury plus N-acetylcysteine occurred at the basolateral membrane. Urinary excretory data also support this notion, in that the rate of excretion of inorganic mercury was greatest in the rats treated with inorganic mercury plus N-acetylcysteine. Our data also indicate that uptake of inorganic mercury in the kidneys of rats treated with inorganic mercury plus cysteine occurred equally at both luminal and basolateral membranes. In addition, the renal uptake of mercury in rats treated with inorganic mercury plus homocysteine occurred predominantly at the basolateral membrane with some component of luminal uptake. The findings of the present study confirm that there are at least two distinct mechanisms involved in the renal uptake of inorganic mercury, with one mechanism located on the luminal membrane and the other located on the basolateral membrane. Our findings also show that cysteine and homologs of cysteine, when coadministered with inorganic mercury, greatly influence the magnitude and/or site of uptake of mercuric ions in the kidney. (J Am Soc Nephrol 9: 551–561, 1998)

A strong body of evidence indicates that the uptake and accumulation of inorganic mercury in the kidneys predominates along the three segments of the proximal tubule (1–8). Two primary mechanisms appear to be involved in the uptake of inorganic mercuric ions along these segments of the nephron. One of the mechanisms is localized on the luminal membrane (9–11) and is dependent on the activity of the brush-border enzyme γ-glutamyltransferase (9), whereas the other mechanism is localized on the basolateral membrane and involves the activity of the organic anion transport system (9–12). However, it is not clear at present what chemical form(s) of inorganic mercury is being transported at both the luminal and basolateral membranes.

Because γ-glutamyltransferase has been shown to catalytically cleave the γ-glutamylcysteine bond on molecules of glutathione that are bound to mercuric ions (13), it is likely that mercuric ions are transported across the luminal membrane of proximal tubular cells as S-conjugates of cysteinylglycine and/or cysteine. In support of the hypothesis that a mercuric conjugate of cysteine is the predominant form in which mercury is taken up along the proximal tubule are in vivo findings from two recent studies. These findings indicate that coadministration of a nontoxic dose of inorganic mercury plus cysteine (in at least a 1:2 ratio) causes enhanced renal uptake mercury (14,15). Additional in vivo data in one of the studies indicate that coadministration of inorganic mercury plus cysteine also causes increased severity of proximal tubular injury in the nephropathy induced by nephotoxic doses of inorganic mercury (15). More specific evidence implicating the luminal transport of mercuric conjugates of cysteine along the proximal tubule comes from a recent in vitro study, in which apparent transport of mercuric conjugates of cysteine was demonstrated in brush-border membrane vesicles (16).

Although the majority of mercury in plasma is bound to large plasma proteins, particularly albumin, there is a sufficiently large enough pool of both reduced glutathione and cysteine in plasma to which inorganic or organic mercuric ions can bind, and form S-conjugates in a nonenzymatic manner based on the affinity of mercuric ions for the free sulfhydryl groups on these two ligands (especially on glutathione) (17). Both reduced glutathione and cysteine are in the plasma of rats at concentrations of approximately 10 μM (18).
It is also possible that there are other mercuric conjugates formed in the plasma or liver that are taken up by the kidneys. For example, homocysteine and N-acetylcysteine are homologs of cysteine that possess a free sulphydryl group to which mercuric ions can bind. Homocysteine forms as a result of the intracellular demethylation of methionine. Since homocysteine is present in the blood, it could serve as a potential ligand for inorganic mercury. Formation of mercuric conjugates of N-acetylcysteine may occur in the liver via the mercapturic pathway. As part of this pathway, molecules of cysteine bound to mercuric ions may become N-acetylated in hepatocytes and then may be transported into the blood as mercuric conjugates of N-acetylcysteine. Because N-acetylcysteine has a negative charge, mercuric conjugates of N-acetylcysteine might be transported by the organic anion transport system.

In the hope of gaining additional information on the mechanisms involved in the renal tubular uptake of inorganic mercury, the disposition of mercury was evaluated and compared in control rats given a nontoxic dose of mercuric chloride and rats coadministered inorganic mercury with cysteine, homocysteine, or N-acetylcysteine in a 1:4 ratio. We mixed and coinjected four times the concentration of thiol relative to the concentration of inorganic mercury to ensure a high probability of forming linear II coordinate covalent bonds between each mercuric ion and two molecules of the respective thiol. Overall, the aim of the present study was to assess the luminal and basolateral contribution to the renal uptake of mercury when mercuric ions are delivered to the nephron in the form of a conjugate of cysteine, homocysteine, or N-acetylcysteine.

The findings from the current study provide new and useful information regarding the chemical forms in which mercuric ions can be transported by proximal tubular epithelial cells. Moreover, the current findings provide information on the mechanisms involved in the renal uptake of inorganic mercury.

Materials and Methods

General Experimental Design

The principal aim of the present study was to test the hypothesis that the renal disposition of mercury is altered significantly when it is coadministered with cysteine, homocysteine, or N-acetylcysteine. The present study was divided into two sets of experiments. In these experiments, the disposition of mercury was evaluated in control rats given a non-nephrotoxic 0.5 μmol/kg intravenous dose of mercuric chloride in normal saline and in rats coadministered intravenously this dose of inorganic mercury with 2.0 μmol/kg cysteine, homocysteine, or N-acetylcysteine. The chemical structure of cysteine, homocysteine, and N-acetylcysteine is presented in Figure 1. All rats used in the present study were male and were of the Sprague Dawley strain. The animals were purchased from Harlan Sprague Dawley (Indianapolis, IN) and weighed 175 to 200 g.

In the first experiment (experiment 1), the disposition (levels) of mercury (in kidneys, liver, and blood) was evaluated 1 h after the 0.5 μmol/kg dose of inorganic mercury had been administered. This allowed us to evaluate the disposition of mercury during a time when maximal rates of renal accumulation of inorganic mercury occur (as determined in our laboratories). To determine the relative contributions of luminal and basolateral uptake of mercury in the kidneys under each treatment condition, the renal disposition of inorganic mercury was assessed and compared in normal rats and rats that had undergone an acute bilateral ureteral ligation. The rationale for this was to generate standard stop-flow conditions in which glomerular filtration was reduced to negligible levels.

In the second experiment (experiment 2), the 24-h disposition (levels) of mercury in the kidneys, liver, and blood and the urinary and fecal excretion of mercury were assessed and compared in control rats injected with the nontoxic dose of mercuric chloride and in rats coinjected with the nontoxic dose of inorganic mercury plus cysteine, homocysteine, or N-acetylcysteine.

Animals and Groups Used in Experiments 1 and 2

Eight groups of randomly selected male Sprague Dawley rats (Harlan Sprague Dawley) were used in experiment 1. There were four
groups of rats with two normal kidneys and four groups of rats that underwent an acute bilateral ureteral ligation. One group of normal rats and one group of rats that underwent bilateral ureteral ligation were paired with respect to receiving one of the four forms of inorganic mercury. In other words, one of each group received an intravenous 0.5 μmol/kg dose of inorganic mercury with or without 2.0 μmol/kg cysteine, homocysteine, or N-acetylcysteine.

In experiment 2, four groups of normal rats were used. The rats received an intravenous 0.5 μmol/kg dose of inorganic mercury with or without 2.0 μmol/kg cysteine, homocysteine, or N-acetylcysteine. The disposition of inorganic mercury in the kidneys, liver, and blood and the urinary and fecal excretion of mercury were determined 24 h after the groups of rats had received their respective injection of mercury.

**Preliminary Studies for Experiment 1**

When attempting to reduce glomerular filtration to negligible levels by ureteral ligation, one must administer a nonreabsorbable solute that can be readily filtered at the glomerulus. This is necessary because the reabsorption of water and solutes continues along the nephron after a ureter has been ligated (14). As a nonreabsorbable solute is initially filtered into the lumen of the proximal tubules of kidneys whose ureter is ligated, the intraluminal osmotic pressure increases until it reaches a level that will essentially stop the reabsorption of solutes and water. This in turn will allow the intraluminal hydrostatic pressure to rise to a point where it is equal or similar to the hydrostatic pressure in the glomerular capillaries, at which time glomerular filtration is diminished to negligible levels (19). The use of mannitol has proven useful for this purpose (19).

**Determination of Maximal Rate of Urine Flow after Administration of Mannitol.** On the basis of previous data obtained from our laboratory (11), maximal rates of urine flow are attained in rats approximately 5 min after the intravenous injection of a 2.0 mmol/kg dose of mannitol. Thus, we chose to perform ureteral ligation 5 min after the injection of mannitol, at which time there should be the highest intraluminal concentration of mannitol along the nephron and collecting duct.

**Determination of Time Required to Achieve Maximal “Stop-Flow” Pressure.** An important consideration when performing ureteral ligation is the time required to achieve a maximal stop-flow pressure, which indicates when the maximal intraluminal hydrostatic pressure is attained and when GFR is at a minimum. On the basis of experiments carried out using a protocol described previously (11), it was decided that inorganic mercury could be administered to animals whose ureters had been ligated 75 min after ligation was performed with the assumption that maximal “stop-flow” conditions would be achieved before the time of injection.

**Experimental Protocol for Experiment 1**

Figure 2 illustrates the experimental protocol used in experiment 1 of the present study. After glomerular filtration was reduced to negligible levels after bilateral ureteral ligation, a 0.5 μmol/kg dose of mercuric chloride (with or without 2.0 μmol/kg l-cysteine, homocysteine, or N-acetylcysteine) was administered (in 2.0 ml/kg normal saline) into the right femoral vein while the rat was under light anesthesia induced by ether. Radioactive inorganic mercury $^{203}$Hg$^{++}$ (specific activity, 15.0 mCi/mg; Buffalo Materials Corp., Buffalo, NY) was added to the injection solution at a concentration to deliver approximately 1 μCi $^{203}$Hg$^{++}$ per animal. After the injection had been administered, the skin over the right femoral vein was approximated with sterile wound clips. Experiment 1 was terminated 1 h after the injection of inorganic mercury. At that time, all animals were anesthetized with a 100 mg/kg dose of sodium pentobarbital, and the acquisition of organs and tissues was carried out to determine the disposition of mercury.

**Experimental Protocol for Experiment 2**

Animals were administered the 0.5 μmol/kg nontoxic dose of inorganic mercury with or without 2.0 μmol/kg l-cysteine, homocysteine, or N-acetylcysteine into the right femoral vein after each animal had been anesthetized lightly with ether as described above. Experiment 2 was terminated 24 h after the injection of inorganic mercury. At the end of the experiment, all animals were anesthetized with a 100 mg/kg dose of sodium pentobarbital, and the acquisition of organs and tissues was carried out to determine the disposition of mercury.

**Acquisition and Handling of Tissues and Organs**

At the end of experiment 1 or 2, a 2.0-ml sample of blood was obtained from the inferior vena cava from each animal after it had been anesthetized with a 100 mg/kg dose of sodium pentobarbital. One milliliter of blood was placed and sealed in a preweighed 12×75-mm polystyrene round-bottomed tube for gamma counting. The other 1 ml was spun down for 10 min at 20,000 × g to separate the cellular fraction of blood from the plasma. Both plasma and cellular fractions were placed individually, and sealed, in counting tubes. After the 2.0-ml sample of blood was obtained, the kidneys and liver were excised and quickly cleared of fat and connective tissue and then weighed. Each of the two kidneys from every animal was cut in half along the transverse plain. One-half of each kidney was placed and sealed in a preweighed polystyrene counting tube. A 3-mm section of
the remaining half of the left kidney and a 3-mm section of the right kidney from the bilaterally ureteral ligated animals were obtained. From this section of tissue, samples of cortex and outer and inner stripes of the outer medulla and inner medulla were obtained and placed in preweighed counting tubes. A 1-g section of liver was also obtained and placed and sealed in a preweighed counting tube. The total volume of blood in each animal was estimated to be 6% of body weight.

In experiment 2, urine and feces were collected for 24 h from each animal. After determining the total volume of urine excreted by each animal, a 1-ml sample was placed and sealed in a preweighed counting tube. The entire amount of feces excreted by each animal was placed in multiple 16-×-100-mm polypropylene counting tubes.

**Determination of the Content of Mercury in Tissues and Organs**

The amount of radioactivity in the samples of tissues, organs, urine, feces, and injection solution (standards) was determined by counting the samples in a 1282 Compugamma CS deep-well gamma spectrometer with a 3" sodium iodide crystal (Wallac, Gaithersburg, MD) operating at a counting efficiency of approximately 50% for 203Hg. The content of mercury in each sample was calculated by dividing the activity (disintegrations per minute [dpm]) in the sample by the specific activity (dpm/nmol) of the injection solution.

**Statistical Analyses**

All values presented are mean ± SEM unless otherwise stated. Because data expressed as a percentage of a total do not fit a normal or Gaussian distribution, all data expressed as a percentage were first normalized using the arcsine transformation before applying any parametric statistical analysis. This transformation takes the arcsine of the square root of the decimal fraction of the percent score. The level of significance for all statistical analyses was chosen a priori to be \( P < 0.05 \). In experiment 1, evaluation of differences between means for any set of data was carried out by applying a two-way ANOVA followed by Tukey’s protected \( t \) test. Statistical analysis of differences between corresponding means for all of the sets of data in experiment 1 was carried out using a one-way ANOVA followed by Tukey’s protected \( t \) test.

**Results**

**Experiment 1**

**Renal Disposition of Mercury in Normal Rats.** Significant differences in the concentration and content of mercury in the kidneys were detected among the groups of normal rats 1 h after the administration of inorganic mercury (Figure 3A). In the control rats that received the 0.5 \( \mu \text{mol/kg} \) dose of inorganic mercury, the renal concentration of mercury was approximately 27% of the dose per gram of tissue, and the total renal burden of mercury was approximately 40% of the administered dose of mercury.

The concentration of mercury in the left kidney and the content of mercury in the total renal mass were greater in rats treated with 0.5 \( \mu \text{mol/kg} \) inorganic mercury plus 2.0 \( \mu \text{mol/kg} \) cysteine than in corresponding control rats treated with the 0.5 \( \mu \text{mol/kg} \) inorganic mercury alone. The levels for the renal concentration and content of mercury in the rats treated with 0.5 \( \mu \text{mol/kg} \) inorganic mercury and 2.0 \( \mu \text{mol/kg} \) homocysteine were greater than those in the rats treated only with inorganic mercury, but were less than those in the rats treated with mercury and cysteine. In the rats treated with 0.5 \( \mu \text{mol/kg} \) inorganic mercury and 2.0 \( \mu \text{mol/kg} \) N-acetylcysteine, the renal concentration and content of mercury were not significantly different from those in the control rats treated only with inorganic mercury.

The concentration of mercury was greater in the cortex and outer stripe of the outer medulla than in any other renal zone in all four groups of normal rats 1 h after the injection of inorganic mercury (Figure 4A). Only in the rats treated with inorganic mercury plus cysteine was the concentration of mercury in the cortex greater than that in the control rats treated with inorganic mercury alone.
In the outer stripe of the outer medulla of control normal rats injected with inorganic mercury only, the concentration of mercury was less than that in the renal outer stripe of the outer medulla of the other three groups of normal rats (Figure 4A). The rats treated with inorganic mercury plus cysteine had the highest levels of mercury in the outer stripe of the outer medulla, followed by the rats that received inorganic mercury plus homocysteine and then the rats treated with inorganic mercury plus N-acetylcysteine. No meaningful significant differences in the concentration of mercury in either the inner stripe of the outer medulla or the inner medulla were detected among the four groups of normal animals.

Renal Disposition of Mercury in Rats Whose Ureters Had Been Ligated. As expected, ureteral ligation had a significant effect on the disposition of mercury in control rats receiving inorganic mercury alone 1 h after the injection of mercury (6). In these control animals, the renal concentration of mercury was approximately 9% of the dose per gram of tissue, and the total renal burden of mercury was approximately 20% of the dose (Figure 3B). These values are significantly different from those in corresponding normal control rats. Ureteral ligation also had significant effects on the renal disposition of mercury in rats treated with inorganic mercury plus cysteine or homocysteine.

Both the renal concentration and content of mercury were greater in the rats that had undergone ureteral ligation and were coadministered inorganic mercury plus cysteine than in the corresponding group given inorganic mercury alone. Moreover, the renal concentration and content of mercury in the rats that had undergone bilateral ureteral ligation and that were coadministered inorganic mercury and homocysteine were greater than those in the corresponding control rats and the corresponding group of rats coadministered inorganic mercury and inorganic mercury.

In the rats that underwent bilateral ureteral ligation and were coadministered N-acetylcysteine and inorganic mercury, the renal concentration and content of mercury were greater than those for any of the other corresponding groups of rats that had undergone bilateral ureteral ligation. Interestingly, the average content of mercury in the total renal mass of the rats that had undergone bilateral ureteral ligation and were treated with inorganic mercury plus N-acetylcysteine was approximately 47% of the administered dose, which was not statistically different from that in the corresponding group of normal rats that were treated in the same way (Figure 3, A and B).

In general, ureteral ligation caused marked reductions in the concentration of mercury in the cortex and outer stripe of the outer medulla during the first hour after treatment, relative to corresponding normal rats (Figure 4B). Rats that had undergone bilateral ureteral ligation and were treated with inorganic mercury plus cysteine had higher concentrations of mercury in their renal cortex and outer stripe of the outer medulla than those in corresponding rats treated with inorganic mercury alone (Figure 4B). In addition, the concentrations of mercury in both the renal cortex and renal outer stripe of the outer medulla in the groups of rats treated with inorganic mercury plus homocysteine or inorganic mercury plus N-acetylcysteine were greater than those in either the rats treated with inorganic mercury plus cysteine or inorganic mercury alone. There was no statistically significant difference in the concentration of mercury in either the renal cortex or renal outer stripe of the outer medulla between the rats treated with inorganic mercury plus homocysteine and the rats treated with inorganic mercury plus N-acetylcysteine. Moreover, there was no significant difference in the concentration of mercury in the outer stripe of the outer medulla between the normal rats treated with inorganic mercury plus N-acetylcysteine and the corresponding rats that underwent bilateral ureteral ligation and were treated with inorganic mercury plus N-acetylcysteine.

As was the case among the groups of normal rats, no meaningful significant differences in the concentration of mercury in either the inner stripe of the outer medulla or the inner medulla were detected among the four groups of rats that had undergone bilateral ureteral ligation.
Content of Mercury in the Liver and Blood of Normal Rats. Approximately 8% of the administered dose of mercury was present in the liver of the control group of normal rats 1 h after the injection of the 0.5 μmol/kg dose of mercuric chloride (Figure 5A). The only significant alteration in the hepatic content of mercury was detected in the group of rats coadministered inorganic mercury and homocysteine. In this group of rats, only approximately 3% of the administered dose of mercury was present in their liver 1 h after treatment.

Approximately 11% of the dose of mercury was present in the blood 1 h after treatment in the normal rats administered the 0.5 μmol/kg dose of inorganic mercury alone (Figure 5A). The amount of mercury in the blood of the other three groups of normal rats was less than one-half of that present in the blood of the normal control group. Among the other three groups of rats, the amount of mercury in the blood was greatest in the group treated with inorganic mercury plus homocysteine.

Of the mercury in blood, only approximately 36% of it was in the plasma (Figure 6A). The remainder was in the cellular fraction of blood, mainly in the erythrocytes. By contrast, between 85 and 90% of the mercury in the blood of the other three groups of normal rats was present in the plasma fraction of blood. No significant differences were detected among the three groups of normal rats that were treated with inorganic mercury plus one of the thiols.

Content of Mercury in the Liver of Rats Whose Ureters Had Been Ligated. A slightly different profile for the hepatic disposition of mercury 1 h after treatment was detected among the four groups of rats that had their ureters ligated. First, with the exception of the groups of rats treated with inorganic mercury alone, the amount of mercury in the liver, between corresponding paired groups of rats that were treated with the same form of mercury, was greater in the group of rats that had their ureters ligated (Figure 5B). Among the four groups of rats that had their ureters ligated, the group of rats treated with inorganic mercury plus N-acetylcycteine had more mercury present in the liver than in any of the other three groups of rats. Interestingly, the amount of mercury in the liver of the group of rats treated with inorganic mercury and homocysteine was lower than that in any of the other corresponding groups of rats.

Bilateral ureteral ligation caused the amount of mercury in the blood to increase, particularly in the rats injected with inorganic mercury alone. In these animals, approximately 20%
of the dose of mercury was present in the blood 1 h after treatment (Figure 5B). Much like in the groups of normal rats, the amount of mercury in the blood of the other three groups of rats that had their ureters ligated was less than one-half of that present in the blood of the control group treated with inorganic mercury alone. Among the other three groups of rats, the amount of mercury in the blood was lowest in the group treated with inorganic mercury plus N-acetylcysteine.

Ureteral ligation did not have a significant effect on the distribution of mercury between the cellular and plasma fractions of blood. For the most part, the same pattern of distribution of mercury between the plasma and cellular fractions of blood detected among the four groups of normal rats was detected among the four groups of rats whose ureters had been ligated (Figure 6B).

**Experiment 2**

**Renal Disposition of Mercury.** Twenty-four hours after treatment, significant differences in the concentration and content of mercury in the kidneys were detected among the four groups normal rats (Figure 7). In the control rats that received the 0.5 μmol/kg dose of inorganic mercury alone, the renal concentration of mercury was approximately 26% of the dose per gram of tissue, and the total renal burden of mercury was approximately 47% of the administered dose of mercury.

The renal concentration of mercury in the rats treated with inorganic mercury plus cysteine or inorganic mercury plus homocysteine was greater than that in the rats treated with inorganic mercury alone (Figure 7). However, among these two comparisons, the total renal burden of mercury was only significantly different between the rats treated with inorganic mercury plus cysteine and the control rats.

By contrast, the renal concentration and the total renal burden of mercury in the rats treated with inorganic mercury plus N-acetylcysteine were less than those in any of the other three groups of rats.

Among the four groups of rats evaluated 24 h after treatment, the only meaningful differences in the cortical concentration of mercury were detected between the rats treated with inorganic mercury plus N-acetylcysteine and the rats treated with inorganic mercury plus homocysteine and the control rats treated only with inorganic mercury (Figure 8). Renal cortical concentrations tended to be lowest in the group treated with inorganic mercury plus N-acetylcysteine.

In the outer stripe of the outer medulla of the rats treated with inorganic mercury plus cysteine, the concentration of mercury was greater than that in any of the other three groups of rats. In the rats treated with inorganic mercury plus homocysteine, the concentration of mercury in the outer stripe of the outer medulla was greater than that in either the rats treated with inorganic mercury plus N-acetylcysteine or the control rats (Figure 8). The concentration of mercury in the outer stripe of the outer medulla tended to be lowest in the rats treated with inorganic mercury plus N-acetylcysteine, although there was no statistically significant difference between in the concentration of mercury in this renal zone between the rats treated with inorganic mercury plus N-acetylcysteine and the control rats treated with only inorganic mercury.

No significant differences in the concentration of mercury in

![Figure 7](image-url)

**Figure 7.** Concentration of mercury (% of administered dose/g tissue) in the left kidney and the content of mercury (% of administered dose) in the total renal mass 24 h after treatment. Values represent mean ± SEM for five animals. *P < 0.05, significantly different from the mean for the corresponding group of control rats treated only with the 0.5 μmol/kg dose of inorganic mercury. **P < 0.05, significantly different from the means for all of the other corresponding groups. *P < 0.05, significantly different from the corresponding mean for the group of rats treated with the inorganic mercury plus cysteine.

![Figure 8](image-url)

**Figure 8.** Concentration of mercury (% of administered dose/g tissue) in the renal cortex, outer stripe of the outer medulla, inner stripe of the outer medulla, and inner medulla 24 h after treatment. Values represent mean ± SEM for five animals. *, **, same as in Figure 7. **P < 0.05, significantly different from the corresponding mean for the control group of rats treated only with inorganic mercury and the corresponding mean for the group treated with inorganic mercury plus cysteine. ***P < 0.05, significantly different from the corresponding mean for the group of rats treated with the inorganic mercury and the corresponding mean for the group of rats treated with inorganic mercury plus homocysteine. **P < 0.05, significantly different from the corresponding mean for the group of rats treated with inorganic mercury plus cysteine and the corresponding mean for the group of rats treated with inorganic mercury plus homocysteine.
either the inner stripe of the outer medulla or inner medulla were detected among the four groups of rats used in this experiment (Figure 8).

**Content of Mercury in the Liver and Blood.** Approximately 7% of the dose of mercury was present in the liver 24 h after treatment in the control rats administered the 0.5 μmol/kg dose (Figure 9). The amount of mercury in the liver of the groups of rats treated with inorganic mercury and one of the three thiols was less than that in the control rats, with the lowest levels detected in the rats treated with inorganic mercury plus cysteine or homocysteine.

The total content of mercury in the blood 24 h after treatment was greatest in the control rats and was lowest in the rats treated with inorganic mercury plus cysteine (Figure 9). Of the mercury present in the blood, far more was present in the plasma fraction of blood in the rats administered inorganic mercury plus one of the thiols (Figure 10). The greatest fraction of blood-mercury present in plasma was detected in the rats treated with inorganic mercury plus cysteine or homocysteine. In these two groups of rats, nearly 70% of the mercury in the blood was present in plasma.

**Urinary and Fecal Excretion of Mercury.** Control rats treated with inorganic mercury alone excreted approximately 5% of the dose of mercury in the urine and slightly more than 6% of the dose of mercury in the feces during the 24 h of the experiment (Figure 11). Rats treated with inorganic mercury plus cysteine or homocysteine excreted much more mercury in the urine. They excreted approximately 11% of the dose in the urine. The rats treated with inorganic mercury plus cysteine excreted about the same amount of mercury in the feces as the control rats, whereas the rats treated with homocysteine excreted only approximately 4% of the dose of mercury in the feces. Urinary excretion of mercury was greatest in the rats treated with inorganic mercury plus N-acetylcysteine. These animals excreted close to 17% of the dose of mercury in the urine in 24 h. Fecal excretion of mercury in these animals was similar to that in the control rats and the rats treated with inorganic mercury plus homocysteine.

**Discussion**

Data obtained from the three groups of rats that were treated with inorganic mercury and cysteine in the present study add to a growing body of evidence implicating the transport of a mercuric conjugate of cysteine and/or glutathione in some aspects of the renal tubular uptake of mercury (9,12,14–16). Despite the fact that the majority of inorganic mercury in plasma is bound to large proteins, both reduced glutathione and cysteine are present in the plasma of rats at concentrations of approximately 10 μM (18). This provides a sufficiently large pool of both reduced glutathione and cysteine to promote the formation of mercuric conjugates of cysteine and/or glutathione in the plasma. Moreover, we recently obtained very preliminary chromatographic data (currently unpublished) indicating that mercuric conjugates of cysteine and glutathione do form in vitro in plasma isolated from the rabbit.
Because the luminal uptake of inorganic mercury has been linked to the activity of \( \gamma \)-glutamyltranspeptidase, which catalytically cleaves the \( \gamma \)-glutamylcysteine bond in molecules of glutathione, it has been postulated that the inorganic mercury is transported across the luminal plasma membrane as a mercuric conjugate of either cysteinylglycine or cysteine (20). Due to the large amount of dipeptidase activity in the luminal membrane of proximal tubular epithelial cells, it seems unlikely that inorganic mercury is transported as a conjugate of cysteinylglycine, at least under normal homeostatic conditions. A more likely scenario would seem to involve the transport of a mercuric conjugate of cysteine, presumably by one of the amino acid transport systems. There is some direct in vitro evidence from brush-border membrane vesicles that mercuric conjugates of cysteine can be transported across the luminal membrane of proximal tubular epithelial cells (16).

A few reports from the 1980s suggest that the luminal uptake of inorganic mercury involves a mercuric conjugate of albumin (4,20,21). Due to the fact that greater than 95% of the mercury in the plasma of blood is bound to large plasma proteins, such as albumin, and that all mammals filter some level of protein at the glomerulus, it is possible (if not likely) that some fraction of inorganic mercury that is filtered into the lumen of the proximal tubule is in the form of a mercuric conjugate of albumin. However, our recent data (unpublished) from isolated proximal tubular segments perfused in vitro with mercuric conjugates of albumin indicate that not very much inorganic mercury is likely taken up along the proximal tubule as a conjugate of albumin. Thus, if some fraction of filtered inorganic mercury is in the form of a mercuric conjugate of albumin, the current evidence on the luminal mechanisms of uptake of mercury would indicate that these mercuric ions are likely exchanged from molecules of albumin to molecules of glutathione in the lumen before being taken up by proximal tubular cells. In support of this notion are our findings (submitted for publication) indicating that the rates of secretion of glutathione into the lumen of all of the segments of the proximal tubule are substantial, especially in the S1 segments. On the basis of these findings, the rates of synthesis and luminal secretion of glutathione are great enough to generate intraluminal concentrations of glutathione that are high enough to promote the exchange of mercuric ions from albumin to glutathione. Obviously, additional studies are needed to test this hypothesis more fully.

Evidence for the basolateral mechanism involving the activity of the organic anion transport system comes mainly from several recent studies in which pretreatment with para-aminobiphenyl was shown to inhibit the renal tubular uptake of administered inorganic mercury (9–12). One conjugate of inorganic mercury that may be transported by the organic anion transporter is the mercuric conjugate of cysteine. In support of this hypothesis is evidence indicating that organic conjugates of cysteine are transported by the organic anion transport system (22). Basolateral uptake of mercuric conjugates of cysteine is also supported indirectly by data from the rats whose ureters had been ligated and were treated with inorganic mercury plus cysteine. Induction of stop-flow conditions (using mannitol injection and bilateral ureteral ligation) caused an approximate one-half reduction in the renal burden of mercury in both control rats treated with 0.5 \( \mu \text{mol/kg} \) inorganic mercury alone and in the rats coadministered this dose of inorganic mercury with 2.0 \( \mu \text{mol/kg} \) cysteine. Moreover, the renal burden of mercury was greater in the animals treated with inorganic mercury plus cysteine than in corresponding control rats treated with inorganic mercury, whereas the relative intrarenal distribution of mercury was similar in both groups of rats. The most reasonable explanation for the findings in these rats treated with inorganic mercury plus cysteine is that more mercuric conjugates of cysteine were made available at the site of the organic anion transporter to promote the uptake of mercury than in the corresponding control rats.

Most (if not all) of the mercury injected into the animals receiving inorganic mercury plus one of the three thiols used in the present study was likely in the form of a linear II coordinate covalent complex consisting of one mercuric ion bound to two molecules of cysteine, homocysteine, or N-acetylhomocysteine (as depicted in Figure 1). This notion is strongly supported by the fact that the content of mercury in the blood was significantly lower, and that partitioning of mercury between the plasma and cellular fractions of blood was greatly different in the animals coadministered mercury with cysteine, homocysteine, or N-acetylhomocysteine relative to that in the animals given inorganic mercury alone. Further support for this notion comes from \(^{13}\text{C}\)-nuclear magnetic resonance data on the association and thermodynamic stability of thiol conjugates of mercury in aqueous solutions (17). For example, it has been demonstrated that when mercuric ions are in aqueous solution with a twofold greater concentration of glutathione molecules, each and every molecule of glutathione becomes incorporated in a thermodynamically stable (throughout a pH range of 1 to 14) linear II coordinate covalent complex consisting of two molecules of glutathione bound to each mercuric ion. Data from the rats that underwent ureteral ligation and were treated with inorganic mercury and homocysteine or N-acetylhomocysteine also tend to indicate that a preponderance of the mercuric conjugates formed in the injection solutions were maintained in the plasma during the first hour after treatment.

Normal rats coadministered inorganic mercury with homocysteine had a renal burden of mercury that was only approximately 14% greater than that in control rats treated with inorganic mercury alone. The difference in the renal burden of mercury between these two groups of rats was, however, statistically significant. Unlike in the rats treated with inorganic mercury plus cysteine, the increased renal burden of mercury in the rats treated with inorganic mercury and homocysteine was due only to increased uptake and/or accumulation of mercury in the outer stripe of the outer medulla. A significant increase in the uptake and/or accumulation of mercury in the outer stripe of the outer medulla was also detected in the rats treated with inorganic mercury plus N-acetylhomocysteine. Despite this increase, the total renal burden of mercury in these animals was not significantly different from that in rats treated with inorganic mercury alone.

In addition to causing significant reductions in the renal...
burden of mercury in rats treated with inorganic mercury plus cysteine, ureteral ligation caused significant reductions in the renal burden of mercury in rats coadministered inorganic mercury plus homocysteine, but they were not as great as those in the group coadministered inorganic mercury with cysteine. Reductions in the uptake and/or accumulation of inorganic mercury in both the cortex and outer stripe of the outer medulla account for the reductions in the renal burden of mercury induced by bilateral ureteral ligation.

Interestingly, bilateral ureteral ligation did not have a significant effect on the renal burden of mercury in the rats coadministered inorganic mercury with N-acetylcysteine. If we assume that glomerular filtration was reduced to negligible levels in the rats that underwent ureteral ligation, the data obtained from the rats coadministered inorganic mercury plus N-acetylcysteine indicate that virtually, if not all, of the mercury taken up by the kidneys in these animals occurred at the basolateral membrane. Data on the urinary excretion of mercury from experiment 2 further support this notion. Rats treated with inorganic mercury and N-acetylcysteine excreted more mercury in the urine than any of the other groups of rats. In fact, they excreted more than threefold the amount of mercury excreted by the control rats treated with inorganic mercury alone. The increased urinary excretion of mercury and presumed lack of luminal uptake of mercury in the rats treated with inorganic mercury and N-acetylcysteine may relate to the negative charge on the molecules. By contrast, this very same negative charge on the molecules of N-acetylcysteine likely is the reason for the avid uptake of inorganic mercury at the basolateral membrane. The implication is that the negative charge on the molecules of N-acetylcysteine bound to mercuric ions promoted the transport of mercuric conjugates of N-acetylcysteine into the proximal tubular cells at the site of the organic anion transporter. In support of this hypothesis is the fact that numerous organic S-conjugates of N-acetylcysteine are known to be transported by the organic anion transporter and that the organic anion transport system has been implicated recently in the basolateral uptake of inorganic mercury (9–12).

Involvement of the organic anion transport system in the basolateral uptake of mercuric conjugates of homocysteine and N-acetylcysteine is also supported indirectly by our dispositional data in normal rats, which show that there is enhanced uptake of mercury almost exclusively in the renal outer stripe of the outer medulla after treatment with inorganic mercury plus homocysteine or N-acetylcysteine. Inasmuch as organic anion transport predominates in the terminal segments of the proximal tubule (23), which are located in the inner cortex and outer stripe of the outer medulla, one might expect increased uptake of inorganic mercury in these regions of the kidney if certain mercuric conjugates were being taken up mainly by the organic anion transporter. This appears to have been the case after treatment with inorganic mercury plus homocysteine or N-acetylcysteine.

Fecal excretion of mercury was altered significantly only in the group of rats treated with inorganic mercury plus homocysteine. In these rats, the fecal excretion of mercury was lower than that in any of the other corresponding groups of rats. It appears that the early diminution in the hepatic uptake of mercury that occurred when inorganic mercury was coadministered with homocysteine caused a decrease in the hepatic biliary elimination of mercury. Additional studies will be necessary to clarify the mechanism(s) involved in the hepatic disposition of mercury when it is in the form of a conjugate with cysteine, homocysteine, or N-acetylcysteine.

Although current experimental evidence suggests that mercuric conjugates of cysteine and glutathione are involved in the luminal and basolateral uptake of inorganic mercury along the proximal tubule after exposure to mercuric chloride, it is clear from the present data that other thiols, especially homologs of cysteine, can influence significantly the manner in which inorganic mercury is handled in the kidneys. For example, our data indicate that when inorganic mercury is bound to homocysteine, there is some uptake of mercury at the luminal membrane, but basolateral uptake of mercury predominates. In addition, our findings indicate that when inorganic mercury is bound to N-acetylcysteine, uptake of mercury occurs almost exclusively at the basolateral membrane. These patterns of uptake and accumulation of mercury are clearly different from those detected in rats treated with inorganic mercury alone or in combination with cysteine. For the purpose of clarification, a diagrammatic model (based on the findings from the present

![Figure 12. Diagrammatic scheme outlining putative mechanisms involved in the transport of mercuric conjugates of cysteine, homocysteine, and N-acetylcysteine. This figure presents the hypothesis that a sodium-dependent transporter (presumably of neutral amino acids) on the luminal membrane and the organic anion transporter on the basolateral membrane are the mechanisms by which mercuric conjugates of cysteine, homocysteine, or N-acetylcysteine are taken up by proximal tubular epithelial cells. It is currently believed that the organic anion transporter is a counter-transporter that is driven in part by the outward movement of α-ketoglutarate from within the proximal tubular epithelial cells. The intracellular concentration of α-ketoglutarate, in turn, is maintained by intracellular generation and basolateral uptake by the sodium-driven dicarboxylic acid cotransporter (24,25). These components have been incorporated into the putative mechanism by which the basolateral uptake of mercuric conjugates of cysteine, homocysteine or N-acetylcysteine occurs.](image-url)
study) summarizing the putative mechanisms involved in the transport and handling of mercuric conjugates or cysteine, homocysteine, and N-acetylcysteine is presented in Figure 12.

The implications of the data on mercuric conjugates of homocysteine or N-acetylcysteine to human health are not known at present. It should be mentioned, however, that elevated levels of homocysteine in the blood have been shown recently to be associated with cardiovascular disease in some individuals. Consequently, these individuals may be more susceptible to the formation of mercuric conjugates of homocysteine in the blood after exposure to inorganic or organic forms of mercury, and thus may be at an increased risk of having a greater renal burden of mercury than normal individuals. It is also not clear whether mercuric conjugates of N-acetylcysteine form in vivo, although the current findings on the renal handling of these conjugates provide interesting new insights into how mercuric ions can be handled in the kidneys.

Acknowledgments

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References