

# Renal Organic Anion Transport System: A Mechanism for the Basolateral Uptake of Mercury-Thiol Conjugates along the Pars Recta of the Proximal Tubule<sup>1</sup>

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The basolateral handling of 20  $\mu\text{M}$  inorganic mercury ( $\text{Hg}^{2+}$ ), in the form of mercuric conjugates of cysteine (Cys), *N*-acetylcysteine (NAC), or glutathione (GSH), was studied in isolated perfused S2 segments of the rabbit proximal tubule. One of the primary aims of the present study was to determine in a direct manner whether basolateral uptake of  $\text{Hg}^{++}$  occurs in the pars recta of the proximal tubule and, more importantly, whether the *p*-aminohippurate-sensitive (PAH) organic anion transport system is involved in this process. Basolateral uptake and accumulation of  $\text{Hg}^{++}$  occurred when the basolateral membrane of the tubular segments was exposed to mercuric conjugates of Cys, NAC, or GSH. Net basolateral uptake of  $\text{Hg}^{++}$  was more than twice as great in the tubules exposed to mercuric conjugates of Cys or NAC than in the tubules exposed to mercuric conjugates of GSH, indicating that mercuric conjugates of Cys or NAC are transported more efficiently than mercuric conjugates of GSH. When PAH (1 mM) was added to the basolateral compartment (bath) surrounding a perfused S2 segment, the net uptake of  $\text{Hg}^{++}$  (in the form of the mercuric conjugates) was reduced by 60–70%. In addition, when glutarate (4 mM), a transportable substrate for both the sodium-dependent dicarboxylate transporter and the dicarboxylate/organic anion exchanger (OAT1), was added to the basolateral compartment, there was a significant reduction in the uptake and accumulation of  $\text{Hg}^{++}$  in the form of mercuric conjugates of Cys. Overall, these data indicate that  $\text{Hg}^{++}$ , in the form of biologically relevant mercuric conjugates of Cys, NAC, or GSH, is taken up significantly at the basolateral membrane of pars recta segments of the proximal tubule, and this uptake is mediated mainly by the actions of the PAH-sensitive organic anion transport system. © 2002 Elsevier Science (USA)

Findings from a number of recent studies indicate that both luminal and basolateral mechanisms participate in the *in vivo*

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uptake of inorganic mercury ( $\text{Hg}^{2+}$ ) along the renal proximal tubule, parts of which are highly sensitive to the toxic effects of this metal (reviewed in Zalups, 2000). Data derived from both whole animal (*in vivo*) and isolated perfused tubule (*in vitro*) studies indicate that the luminal mechanisms involved are largely dependent on the actions of the brush-border membrane enzymes  $\gamma$ -glutamyltransferase and cysteinylglycinase (Tanaka *et al.*, 1990; Tanaka-Kagawa *et al.*, 1993; Zalups, 1995; Cannon *et al.*, 2000) and the activities of both sodium-dependent and sodium-independent amino acid transport systems (Cannon *et al.*, 2000, 2001). Moreover, recent isolated perfused tubule findings of Zalups and Barfuss (Cannon *et al.*, 2000; 2001) indicate that much of this luminal uptake of  $\text{Hg}^{++}$  may involve a mechanism of molecular homology or “mimicry,” whereby the mercuric conjugate dicysteinymercury (Cys–Hg–Cys) acts as molecular homolog or “mimic” of cysteine at the site of amino acid transporters involved in the luminal absorption of this amino acid.

By contrast, there are only indirect lines of evidence indicating that basolateral uptake of  $\text{Hg}^{++}$  occurs along the proximal tubule *in vivo*. These lines of evidence come primarily from whole animal studies. One line of data shows that, following unilateral or bilateral ureteral ligation, significant levels of uptake and accumulation of  $\text{Hg}^{++}$  continue to occur in the kidneys of rats injected intravenously with a nontoxic dose of  $\text{Hg}^{++}$  (Zalups, 1995; Zalups and Minor, 1995). Another set of data shows the renal tubular uptake and net accumulation of administered  $\text{Hg}^{++}$  are reduced greatly in rats pretreated with *p*-aminohippurate (PAH) (Zalups, 1995, 1998a,b; Zalups and Barfuss, 1995; Zalups and Minor, 1995), which is a well-characterized competitive substrate for the dicarboxylate/organic anion exchanger (OAT1) (Dantzler, 1996; Dantzler *et al.*, 1995; Pritchard and Miller, 1993; Roch-Ramel *et al.*, 1992). Moreover, it has been shown that both ureteral ligation and pretreatment with PAH have an additive effect in reducing the renal tubular uptake and accumulation of  $\text{Hg}^{++}$  in rats. Furthermore, data from the study of Zalups and Barfuss, (1998a) show that pretreatment of rats with small, five- to six-carbon aliphatic dicarboxylates (such glutarate or adipate)

inhibits significantly the renal tubular uptake of administered  $\text{Hg}^{++}$ . The significance of these findings comes from the fact that these dicarboxylates are competitive substrates at both the sodium-dependent dicarboxylate transporter and the dicarboxylate/organic anion exchanger (OAT1) of the basolateral organic anion transport system that is located exclusively in proximal tubular segments. Normally, the sodium-dependent dicarboxylate transporter in the basolateral membrane recycles  $\alpha$ -ketoglutarate back into proximal tubular epithelial cells to maintain the intracellular concentration of this dicarboxylate high enough to efficiently drive the activity of the dicarboxylate/organic anion exchanger (OAT1) (Dantzler, 1996; Dantzler *et al.*, 1995; Pritchard and Miller, 1993; Roch-Ramel *et al.*, 1992). The *in vivo* studies of Zalups, (1998a,b) and Zalups and Barfuss, (1998b) also demonstrate that mercuric conjugates of cysteine (Cys), *N*-acetylcysteine (NAC), and glutathione (GSH) are likely transportable species of  $\text{Hg}^{++}$  at the basolateral membrane of proximal tubular epithelial cells (Zalups, 1998a,b; Zalups and Barfuss, 1998b).

One of the first lines of *in vitro* data supporting the hypothesis that inward basolateral flux of specific mercuric conjugates occurs along the renal proximal tubules comes from the study of Zalups and Lash (1997). They demonstrated that mercuric conjugates of Cys (perhaps GSH) are transported into basolateral membrane vesicles isolated from the renal cortex of rats. Unfortunately, because of high levels of nonspecific binding in the membrane vesicles, substantive conclusions could not be made about the exact nature of how  $\text{Hg}^{++}$  is handled at the basolateral membrane of proximal tubular epithelial cells *in vivo*.

Notwithstanding all of the circumstantial evidence implicating a basolateral mechanism in the proximal tubular uptake and accumulation of  $\text{Hg}^{++}$ , there has been a lack of direct evidence establishing that basolateral uptake of specific mercuric-thiol conjugates occurs in the epithelial cells lining the proximal tubule and that the PAH-sensitive organic anion transport system is involved in this process. Therefore, with the aid of the isolated perfused tubule technique, we were able to test, in the most direct manner to date, the hypothesis that mercuric conjugates of Cys, NAC, or GSH are transported into the epithelial cells of isolated perfused S2 segments of the rabbit proximal tubule when the conjugates are applied directly to the basolateral membrane of these cells. Moreover, by using competitive substrates of the dicarboxylate/organic anion exchanger (OAT1) and the sodium-dependent dicarboxylate transporter, we were able to test directly the hypothesis that the organic anion exchanger (OAT1) plays a significant role in mediating basolateral uptake of  $\text{Hg}^{++}$  (in the form of mercuric conjugates of biologically relevant low-molecular-weight thiols) along pars recta of the proximal tubule.

The S2 segment was chosen for study because this is the portion of the proximal tubule that makes up the major component of the pars recta, which begins in the outer- to midcortex and extends down to the corticomedullary junction (Kriz

and Bankir, 1988). More importantly, this segment is one of the main portions of the proximal tubule in which  $\text{Hg}^{++}$  exerts its toxic effects (Zalups, 2000) and is also the portion of proximal tubule in which the activity of the organic anion transport system is the greatest (Roch-Ramel *et al.*, 1992).

## MATERIALS AND METHODS

### Animals

Female, New Zealand White, specific pathogen-free, rabbits (purchased from Myrtle's Rabbit Farm, Thompson Station, TN) weighing 1.0–1.5 kg were used in the present study. Prior to experimentation, the rabbits were maintained on regular rabbit chow and given water *ad libitum*. All experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals. A minimum of two rabbits was used for each experimental condition studied.

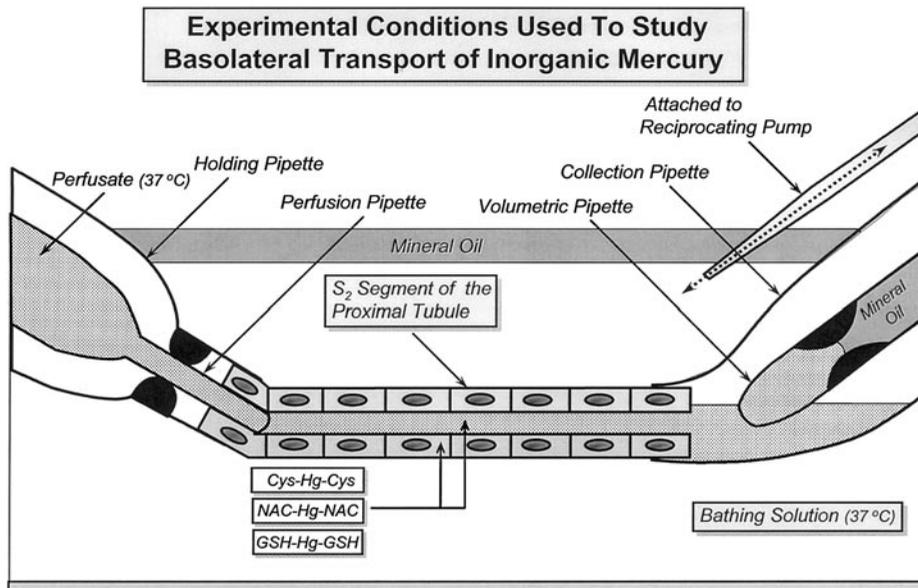
### Procedure for Obtaining S2 Segments of the Proximal Tubule

On each day of experimentation, a rabbit was anesthetized with a combination of 33 mg/kg ketamine and 33 mg/kg xylazine, which were purchased from Butler Chemical Co. (Bedford, OH). After induction of anesthesia (as determined by corneal reflex), the abdomen was opened and the kidneys were removed and placed in a cold (4°C) aqueous phosphate–sucrose buffer solution. The phosphate–sucrose buffer solution contained 125 mM sucrose, 13.3 mM  $\text{NaH}_2\text{PO}_4$ , and 56 mM  $\text{Na}_2\text{HPO}_4$ , and the pH of the solution was adjusted to 7.4 with 1.0 M NaOH. The kidneys were then sliced quickly into 1- to 2-mm coronal sections with a single-edge razor blade. The tissue sections were stored in the same phosphate–sucrose buffer solution. S2 segments of the proximal tubule, which were identified as straight portions of the proximal tubule that spanned the entire thickness of the cortex, were dissected from these slices under a dissecting microscope for the next 6 to 8 h. As alluded to above, this particular segment of the proximal tubule was chosen for study because (1) it can be dissected readily from the rabbit kidney without the use of collagenase, (2) it makes up the major portion of the pars recta of the proximal tubule (which accumulates  $\text{Hg}^{++}$  avidly and is involved in the nephropathy induced by this toxic metal), and (3) it is the segment of the proximal tubule in which the activity of organic anion transport system is greatest (Roch-Ramel *et al.*, 1992).

### Method for Studying Bath-to-Lumen Flux in Isolated S2 Proximal Tubular Segments

As in lumen-to-bath flux studies, each dissected tubule was transferred to a Lucite perfusion chamber and was suspended between two sets of pipettes. One set of pipettes was used to perfuse the suspended tubule, while the other set was used to collect the perfused luminal fluid. Each tubule was allowed to warm to 37°C for 5 min prior to beginning an experiment. Perfusion rates were maintained on average between 7 and 10 nL/min by application of constant hydrostatic pressure. Due to differences in the diameter of the perfusion pipettes used, the hydrostatic pressures needed to perfuse S2 segments of the proximal tubule at rates between 7 and 10 nL/min varied between 15 and 50 mm Hg. It should be stressed that most of the drop in pressure occurs at the tip of the perfusion pipette. In addition, each perfused tubule was monitored for changes in tubular diameter resulting from abnormally high intraluminal pressures. The perfused fluid was collected from the lumen into a constant volume pipette (designed to collect accurately 30–50 nL of fluid).

The total volume of solution bathing the basolateral surface of each perfused tubular segment (0.3 mL) was covered with a layer of lightweight mineral oil to prevent evaporation. Unstirred layers around the perfused tubule were prevented from occurring by continuously mixing the bathing solution with a reciprocating pump. The bathing solution was drawn up into, and expelled from, the pump through a piece of polyethylene tubing, which was placed



**FIG. 1.** The basic apparatus and conditions used to study the basolateral transport of mercuric conjugates of cysteine (Cys), *N*-acetylcysteine (NAC), or glutathione (GSH) in isolated perfused S2 segments from the rabbit proximal tubule. Once an S2 segment (generally around 1 mm in length) had been dissected free from a sagittal section of the rabbit kidney, it was transferred to the perfusion chamber (in a transfer pipette) where it was submerged into a basic electrolyte solution (that was maintained at 37°C throughout each experiment) below a layer of lightweight mineral oil. Extreme care is required to move each dissected tubule through the layer of mineral oil, which is used to prevent evaporation of the aqueous fluid in the chamber. The basic electrolyte solution used to bathe the tubules contained 20  $\mu\text{M}$  mercuric chloride or 20  $\mu\text{M}$  mercuric ions in the presence of 60–80  $\mu\text{M}$  Cys, NAC, or GSH. The 3:1 or 4:1 ratio of thiol to mercuric ions ensured the formation of thermodynamically stable dithiol conjugates of mercury at a concentration of 20  $\mu\text{M}$  (Rabenstein, 1989). Once the tubule had passed through the oil interface, it was placed to rest on the bottom of the perfusion chamber, which was made from a glass coverslip for microscopic slides. After the dissected tubular segment was placed on the bottom of the perfusion chamber, it was suspended between two sets of two fabricated pipettes in the following manner: (1) the tubule was first attached to the holding pipette, (2) the perfusion pipette was then carefully moved down the center of the holding pipette until the tip of the perfusion pipette had entered into the lumen of the tubule, and (3) once the perfusion pipette was in place, the collection pipette was attached quickly to the opposite end of the tubule. When all of the pipettes were in place, the perfusion process was initiated. All S2 segments were perfused through the lumen with the same basic electrolyte solution (which did not contain any  $\text{Hg}^{++}$ ) at a rate of approximately 7–10 nL min. The tip of an additional pipette that was attached to a reciprocating pump was placed through the oil–water interface so as to thoroughly mix the bathing solution (0.3 mL) surrounding the perfused S2 segment during each experiment. By vigorously mixing the bathing solution, unstirred layers were prevented from forming around the perfused tubular segment.

through the layer of oil into the aqueous bathing solution. Approximately 50  $\mu\text{L}$  of bathing solution were repeatedly withdrawn and then injected back into the bath. This was sufficient to produce a vigorous mixing of the bathing solution. Figure 1 displays the basic apparatus and conditions used to study the basolateral transport of  $\text{Hg}^{++}$  in isolated S2 segments of the proximal tubule. For additional details on the methods used to isolate and perfuse segments of the proximal tubule of the rabbit refer to Zalups and Barfuss (1996).

*Note:* Among the techniques used to study transport in isolated perfused tubules, the technique used to study bath-to-lumen flux of solutes is the most difficult, mainly because of the difficulties associated with getting an isolated nephron segment in and out of the perfusion chamber through the oil–water interface.

#### Composition of Perfusing and Bathing Solutions.

In all of our experiments, the perfusing and bathing solutions consisted of simple electrolyte solutions. The perfusing solution contained the following: 145 mM  $\text{Na}^+$ , 140 mM  $\text{Cl}^-$ , 5 mM  $\text{K}^+$ , 2.5 mM  $\text{Ca}^{2+}$ , 1.2 mM  $\text{Mg}^{2+}$ , 1.2 mM  $\text{SO}_4^{2-}$ , 2 mM  $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$ , 1 mM D-glucose, and 0.5 mM glutamine and the pH was adjusted to 7.4 with 1 N NaOH. In order to evaluate the cytotoxic effects of  $\text{Hg}^{++}$ , we placed the vital dye FD&C green No. 3 (MW = 809 Da) in the perfusate at a concentration of 250 nM. The final osmolality of the

perfusing and bathing solutions was adjusted to 290 mOsmol  $\text{kg}^{-1}$   $\text{H}_2\text{O}$  using doubly distilled and deionized water. In all experiments, the basic composition of the bathing solution was the same as that for the perfusing solution. [ $^3\text{H}$ ]L-Glucose (50  $\mu\text{Ci}/\text{ml}$ , SA = 58.8 mCi/mg) was used as a volume marker in all experiments and was added to the bathing solution. Experimental solutes, such as inorganic mercury (containing  $^{203}\text{Hg}^{2+}$ , 18  $\mu\text{Ci}/\mu\text{g}$ ), Cys, cystine, NAC, GSH, PAH, or glutarate, were added to the bathing solutions in particular experiments. Refer to the Experimental Design section for additional details on perfusion conditions.

#### Production of $^{203}\text{Hg}^{2+}$

Three milligrams of mercuric oxide ( $\text{HgO}$ ) containing the stable isotope  $^{200}\text{Hg}^{++}$  and enriched  $^{202}\text{Hg}^{++}$  (target) were weighed and doubly-sealed in quartz tubing. The actual isotopic composition of the mercuric oxide used was <0.05%  $^{196}\text{Hg}$ , 1.5%  $^{198}\text{Hg}$ , 2.82%  $^{199}\text{Hg}$ , 4.24%  $^{200}\text{Hg}$ , 3.11%  $^{201}\text{Hg}$ , 86.99%  $^{202}\text{Hg}$ , and 1.34%  $^{204}\text{Hg}$ . The doubly encapsulated target was sent to the Missouri University Research Reactor (MURR) facility to be irradiated (by neutron activation) for a period of 4 weeks. After receiving the irradiated target from the MURR facility, the target was placed in protected storage for a period of 10 days to allow for the isotopic decay of the newly formed  $^{197}\text{Hg}^{++}$ . Subsequently, the two quartz tubes enclosing the target were broken in half.

The target was removed from the quartz tubing with four 50- $\mu\text{L}$  rinses of 1 N HCl. All four rinses were placed and sealed (temporarily) in a single 1.7-mL polypropylene vial. A sample of the solution was then used to determine the precise solid content of Hg in the isotopic solution using plasma-coupled elemental mass spectrometry. The radioactivity of the solution was determined with a Wallac (Gaithersburg, MD), Wizard 3<sup>rd</sup> 1480 Automatic Gamma Counter, which allows one to determine the activities of up to 10 different isotopes simultaneously. The counting efficiency for  $^{203}\text{Hg}$  in the Wallac gamma counter is approximately 50%. The specific activities of the  $^{203}\text{Hg}^{++}$  used in the present study ranged between 12 and 18 mCi/mg Hg.

#### Collecting Samples

Samples of luminal fluid exiting from a perfused tubular segment (collected) were collected from each perfused tubule in order to measure rates of bath-to-lumen flux ( $J_{\text{A-BL}}$ ;  $\text{fmol} \times \text{mm} (\text{tubular length})^{-1} \times \text{min}^{-1}$ ) of  $\text{Hg}^{++}$  and the volume marker. The time required to fill the constant volume pipette was used to calculate the volume collection rate ( $\text{nL} \times \text{min}^{-1}$ ).

#### Harvesting Perfused Tubular Segments at the End of an Experiment

In order to calculate the cellular content of  $\text{Hg}^{++}$  in the isolated perfused S2 segments of the proximal tubule, it was necessary to harvest the perfused tubule at the end of each experiment. The tubular segment was harvested (while it was being perfused) and it was removed out of the bathing solution and was first washed in 10  $\mu\text{L}$  of cold (5°C) perfusion buffer and then was placed in 10  $\mu\text{L}$  of 3% (w/v) trichloroacetic acid (TCA). After a few minutes, the tubular segment was removed from the TCA solution. The TCA-soluble fraction and TCA-insoluble fractions of a tubular segment were each placed individually in a vial of scintillation fluid and were analyzed (by standard isotopic methods) later for the content of both  $^{203}\text{Hg}^{++}$  and the volume marker [ $^3\text{H}$ ]-glucose. All tubules were removed from the pipettes after 30 min of perfusion. This permitted us to assess and compare more accurately the basolateral uptake and tubular accumulation of  $\text{Hg}^{++}$  over time among the treatment conditions studied.

#### Experimental Design

The present investigation consisted of three separate sets of experiments. In the first set of experiments, bath-to-lumen flux and cellular accumulation of mercuric ions ( $\text{Hg}^{++}$ ) were evaluated in isolated S2 segments perfused through the lumen with the basic electrolyte solution while they were exposed to 20  $\mu\text{M}$   $\text{Hg}^{++}$  and 60 or 80  $\mu\text{M}$  Cys, NAC, or GSH at their basolateral membrane. The presence of a 3 or 4:1 ratio of thiol to  $\text{Hg}^{++}$  ensured the formation of linear II coordinate covalent bonds (thiol-Hg-thiol) between each mercuric ion and two molecules of the respective thiol. Based on the affinity and thermodynamic stability of the bonding between the mercuric ions and the thiol molecules in an aqueous environment (Rabenstein, 1989), one can assert confidently that the basolateral membrane of each perfused S2 segment was exposed to 20  $\mu\text{M}$  mercuric S-conjugates of the thiols being studied (as well as unbound thiol molecules). The primary rationale for evaluating the basolateral transport of mercuric conjugates of Cys, NAC, and GSH is that findings from animal studies and various *in vitro* experiments have implicated these conjugates in the proximal tubular uptake of  $\text{Hg}^{++}$  (Lash *et al.*, 1998; Zalups, 1998a,b, 2000; Zalups and Barfuss, 1998a,b; Zalups and Lash, 1997). These initial experiments represent the first attempts to demonstrate directly, in an intact tubular epithelium, that basolateral uptake of specific mercuric conjugates occurs in proximal tubular epithelial cells. A major advantage of using the isolated perfused tubule technique is that the basolateral transport of specific solutes can be studied and characterized in intact proximal tubular segments under biophysical conditions (such as the rates of flow of luminal fluid and intraluminal hydrostatic pressures) similar to those present *in vivo*.

In the second set of experiments, the effects of 2 mM PAH on the basolateral uptake and accumulation of  $\text{Hg}^{++}$  in the form of mercuric conjugates of Cys, NAC, and GSH were assessed. PAH was chosen because it is an effective

substrate and inhibitor of the dicarboxylate/organic anion exchanger (Dantzler, 1996; Dantzler *et al.*, 1995; Pritchard and Miller, 1993; Roch-Ramel *et al.*, 1992) and the concentration of 2 mM was chosen because the affinity and  $K_m$  of PAH for the organic anion transport system in proximal tubular segments is approximately 100  $\mu\text{M}$  (Roch-Ramel *et al.*, 1992; Dantzler *et al.*, 1995).

Finally, in the last set of experiments, we tested the hypothesis that glutarate can *cis*-inhibit the basolateral uptake of the mercuric conjugate dicysteinymercury (Cys-Hg-Cys) at the site of the organic anion transport system. The rationale for this hypothesis comes from *in vivo* experiments in which we demonstrated that five- and six-carbon dicarboxylates (such as glutarate and adipate), known to compete for entry into proximal tubular epithelial cells at the site of the organic anion transporter, *cis*-inhibit the basolateral uptake of  $\text{Hg}^{++}$  along proximal tubular segments. We chose the concentration of 4 mM for glutarate on the basis of findings that had been obtained previously from preliminary experiments with isolated perfused proximal tubular segments.

#### Assessment of Cellular and Tubular Pathology

During each experiment, the perfused tubule was observed microscopically throughout the entire perfusion process in order to detect any cellular pathology. Typical pathological changes induced by  $\text{Hg}^{++}$  include cellular swelling, cytoplasmic vacuolization, shedding of brush-border membrane, blebbing of the apical cytoplasm, and uptake of the vital dye FD&C green (when the epithelial cells die). A control set of five S2 segments of the proximal tubule were used to document the severity of the rapidly progressing pathological changes induced during basolateral exposure to 20  $\mu\text{M}$   $\text{Hg}^{++}$  in the absence of organic molecules that bind  $\text{Hg}^{++}$ .

#### Calculations

**Bath-to-lumen flux.** In bath-to lumen flux experiments, we determined the rate at which  $\text{Hg}^{++}$  appeared in the luminal fluid from the bathing fluid. This appearance flux ( $J_{\text{A-BL}}$ ;  $\text{fmol} \times \text{min}^{-1} \times \text{mm} (\text{tubular length})^{-1}$ ) was calculated by measuring the rate of appearance of the solute in the lumen when it was placed in the bathing solution only. Eq. (1) was used for this calculation:

$$J_{\text{A-BL}} = (\text{dpm}_{\text{Hg}} \div (\text{sa}_{\text{Hg}} \times T)) \div L, \quad (1)$$

where  $\text{dpm}_{\text{Hg}}$  represents the amount of disintegrations per minute of  $^{203}\text{Hg}^{++}$  that appeared in the luminal fluid,  $\text{sa}_{\text{Hg}}$  represents the specific activity ( $\text{dpm fmol}^{-1}$ ) of the  $^{203}\text{Hg}^{++}$ ,  $T$  is the time (in minutes) required to collect the sample, and  $L$  is the length (in mm) of the tubular segment.

**Cellular content of  $\text{Hg}^{++}$ .** The content of  $\text{Hg}^{++}$  ( $\text{Hg}_{\text{Tubule}}$ ) in a perfused tubular segment was calculated using Eq. (2):

$$\text{Hg}_{\text{Tubule}} = (\text{dpm}_{\text{Hg}} \div \text{sa}_{\text{Hg}}) \div L, \quad (2)$$

where  $\text{dpm}_{\text{Hg}}$  represents the disintegrations per minute of  $^{203}\text{Hg}^{++}$  in the perfused tubular segment (both TCA-soluble and TCA-insoluble fractions),  $\text{sa}_{\text{Hg}}$  represents the specific activity ( $\text{dpm fmol}^{-1}$ ) of  $^{203}\text{Hg}^{++}$  taken from standards, and  $L$  is the length of the perfused tubular segment in millimeters. All cellular content was factored by 30 min, which is the length of each perfusion experiment.

As part of the determination of the amount of  $\text{Hg}^{++}$  that accumulates in a perfused segment, one has to account for the amount of  $\text{Hg}^{++}$  that is nonspecifically bound or attached to the basolateral surface of a tubular segment during the extraction of the tubule (from the bathing chamber) at the end of each experiment. This determination was made from the amount of volume marker attached to the tubular segment. The bathing fluid attached to a perfused tubule during the extraction of a tubular segment contains amounts of volume marker and  $\text{Hg}^{++}$  proportional to those in the bathing solution. The calculated amount of  $\text{Hg}^{++}$  that was nonspecifically bound to a perfused segment was subtracted from the total amount of  $\text{Hg}^{++}$  present in the tubular segment. Generally, if the extraction process is performed properly, less than

1% of the total amount of  $\text{Hg}^{++}$  associated with a tubular segment can be attributed to nonspecific attachment of bathing fluid to the basolateral surface.

#### Intercellular Leak of the Volume Marker

Since the epithelial cells in various segments of the nephron permit the intercellular passage of certain solutes (through junctional complexes) to varying degrees, it is important to determine, and to factor in, the magnitude of intercellular leak of the volume marker when computing the rates of transport of a solute in a perfused tubular segment. Volume markers are mainly used in isolated perfused tubules to determine the fluid volume of a compartment, such as the luminal compartment. This allows one to determine accurately the concentration(s) of a solute in that compartment. The rate of bath-to-lumen leak of fluid ( $L_{\text{VM}}$ ,  $\text{nL min}^{-1}$ ) in a tubular segment was measured by the rate of appearance of the volume marker in the luminal fluid. This was determined using Eq. (3).

$$L_{\text{VM}} = \text{dpm}_{\text{VM}} \div ([\text{VM}] \times T), \quad (3)$$

where  $\text{dpm}_{\text{VM}}$  represents the number of disintegrations per minute of the radioisotope on the volume marker that appears in the luminal solution,  $[\text{VM}]$  is the concentration of the volume marker in the luminal fluid expressed as the radioactivity of the volume marker per unit liquid volume ( $\text{dpm nL}^{-1}$ ), and  $T$  is the time (in minutes) required to collect the sample of luminal fluid.

#### Statistical Analysis

Data for each parameter assessed in the first set of experiments were first analyzed with a one-way analysis of variance (ANOVA). When significant  $F$  values were obtained with the ANOVA, Tukey's multiple comparison procedure was used to detect significant differences among the means. A two-tailed Student's  $t$  test was used in the second and third sets of experiments to evaluate statistically the differences between corresponding pairs of means for experimental and control data. The level of significance for all statistical analyses performed was chosen *a priori* to be  $p < 0.05$ .

## RESULTS

#### Visual Observations

In the control set of isolated perfused S2 segments, basolateral exposure to  $20 \mu\text{M Hg}^{++}$  (in a basic electrolyte solution not containing any proteins or sulfhydryl-containing molecules) induced rapidly severe pathological changes along the entire length of tubule. The initial changes consisted of severe cellular swelling, which occurred within the first 5 min of exposure. The swelling became so severe that the lumen of the perfused segments became occluded. Shedding of the brush-border membrane, cytoplasmic blebbing, and cellular death ensued subsequently. In general, uptake of the FD&C green dye, indicating cellular death, occurred within the first 15–20 min of basolateral exposure.

By contrast, when S2 segments were exposed to  $20 \mu\text{M Hg}^{++}$  and  $60\text{--}80 \mu\text{M Cys}$ ,  $\text{NAC}$ , or  $\text{GSH}$  at their basolateral membrane, very few pathological changes were detected during the 30-min experimental period. Occasionally, slight swelling affecting the entire length of a perfused tubule was noted. This was particularly the case in the tubules exposed to  $\text{Hg}^{++}$  and  $\text{Cys}$  or  $\text{NAC}$ . Virtually no pathology was detected in the S2 tubular segments exposed to  $\text{Hg}^{++}$  and  $\text{GSH}$ .

When  $2 \text{ mM PAH}$  was added to the bathing solution containing  $20 \mu\text{M Hg}^{++}$  and  $60\text{--}80 \mu\text{M Cys}$ ,  $\text{NAC}$ , or  $\text{GSH}$ , no demonstrable tubular or cellular pathology could be detected in any of the tubular segments. Moreover, the tubular segments appeared very healthy and pristine morphologically.

Very little to no pathology was detected in any of the tubules perfused under conditions in which  $4 \text{ mM glutarate}$  was present in the bathing solution.

#### Bath-To-Lumen Flux Data

Under all experimental conditions studied, the rate of flux (transport) of  $\text{Hg}^{++}$  from the bathing solution into the luminal compartment of the perfused S2 segments (appearance flux ( $J_{\text{A-BL}}$ )) was negligible (not statistically different from zero). These data indicate that, regardless of the chemical form of  $\text{Hg}^{++}$  in the basolateral compartment, once the  $\text{Hg}^{++}$  entered into the intracellular compartment of the isolated perfused S2 segment, negligible amounts of  $\text{Hg}^{++}$  were transported out of the epithelial cells into the lumen.

#### Intercellular Leak

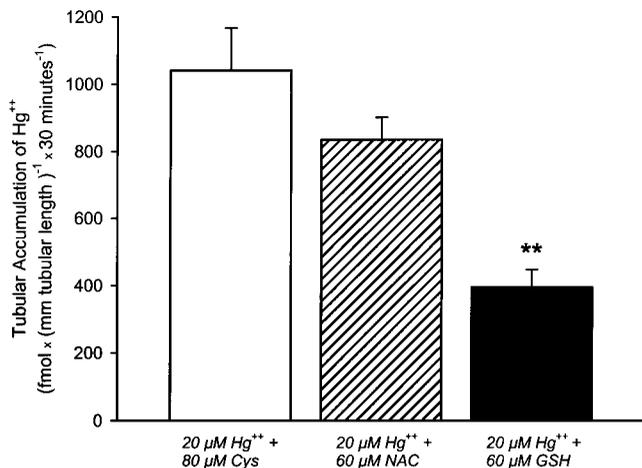
Intercellular leak of the volume marker ( $[^3\text{H}]\text{L-glucose}$ ) was also very low to negligible in all of the experiments, which validates the transport data collected under all of the conditions studied.

#### Bath-to-Cell Flux and Cellular Accumulation Data

Inasmuch as transepithelial bath-to-lumen flux ( $J_{\text{A-BL}}$ ) was essentially nonexistent in any of the perfused tubular segments, the net rate of accumulation of  $\text{Hg}^{++}$  can be translated to a rate of basolateral flux ( $J_{\text{A-Cell}}$ ) of  $\text{Hg}^{++}$  into the proximal tubular epithelial cells. Moreover, virtually all of the  $\text{Hg}^{++}$  detected in a perfused tubular segment was associated with the TCA-insoluble fraction, indicating that most of the intracellular  $\text{Hg}^{++}$  was mainly bound to cellular proteins.

*Influence of thiol-conjugation on basolateral flux and accumulation of  $\text{Hg}^{++}$ .* During the experimental period of basolateral exposure, the rates of uptake and net accumulation of  $\text{Hg}^{++}$  were significantly greater in the S2 segments exposed to  $20 \mu\text{M Hg}^{++}$  and  $80 \mu\text{M Cys}$  or  $60 \mu\text{M NAC}$  than in the corresponding tubules exposed to  $20 \mu\text{M Hg}^{++}$  and  $60 \mu\text{M GSH}$  (Fig. 2). In the tubules exposed to  $\text{Hg}^{++}$  and  $\text{Cys}$  or  $\text{NAC}$ , the rates of basolateral influx averaged approximately  $1040$  and  $840 \text{ fmol} \times \text{mm (tubular length)}^{-1} \times 30 \text{ min}^{-1}$ , respectively. By contrast, the rates of influx in the tubules exposed to  $20 \mu\text{M Hg}^{++}$  and  $60 \mu\text{M GSH}$  at their basolateral membrane averaged about only  $390 \text{ fmol} \times \text{mm (tubular length)}^{-1} \times 30 \text{ min}^{-1}$ .

*Effects of PAH on basolateral flux and accumulation of  $\text{Hg}^{++}$ .* The net rates of basolateral uptake and accumulation of  $\text{Hg}^{++}$  were significantly different between the group of tubules exposed to  $20 \mu\text{M Hg}^{++}$  and  $80 \mu\text{M Cys}$  and the group of tubules exposed to  $20 \mu\text{M Hg}^{++}$ ,  $80 \mu\text{M Cys}$ , and  $2 \text{ mM}$



**FIG. 2.** Rate of basolateral accumulation ( $\text{fmol mm} \times (\text{tubule length})^{-1} \times 30 \text{ min}^{-1}$ ) of inorganic mercuric ions ( $\text{Hg}^{++}$ ) in S2 segments of the rabbit proximal tubule exposed to  $20 \mu\text{M Hg}^{++}$  and 60 or 80  $\mu\text{M}$  cysteine (Cys), *N*-acetylcysteine (NAC), or glutathione (GSH) at their basolateral membrane. All tubular segments were perfused through the lumen with a basic electrolyte solution that did not contain any mercury. Inasmuch as the net rate of flux of  $\text{Hg}^{++}$  ( $\text{fmol mm} \times (\text{tubular length})^{-1} \times \text{min}^{-1}$ ) from the bathing solution into the tubular lumen ( $J_{\text{A-BL}}$ ) was statistically negligible, the rate of accumulation approximated the rate at which  $\text{Hg}^{++}$  was taken up at basolateral membrane. Each value represents the mean  $\pm$  SE for data obtained from six to eight tubular segments. \*\*Significantly different ( $p < 0.05$ ) from the other two means.

PAH (Fig. 3A). The rates of basolateral flux of  $\text{Hg}^{++}$  in the tubules exposed to PAH were on average about 68% lower than those in the corresponding tubules not exposed to PAH.

Similar differences in the basolateral flux and accumulation of  $\text{Hg}^{++}$  were detected between the group of tubules exposed to  $20 \mu\text{M Hg}^{++}$  and 60  $\mu\text{M}$  NAC and the group of tubules exposed to  $20 \mu\text{M Hg}^{++}$ , 60  $\mu\text{M}$  NAC, and 2 mM PAH (Fig. 3B). The mean rate of inward basolateral flux of  $\text{Hg}^{++}$  in the tubules exposed to PAH was about 60% lower than that in the corresponding group of tubules not exposed to PAH.

Statistically significant differences in the net rate of basolateral uptake and accumulation of  $\text{Hg}^{++}$  were also detected between the group of tubules exposed to  $20 \mu\text{M Hg}^{++}$  and 60  $\mu\text{M}$  GSH and the group exposed to  $20 \mu\text{M Hg}^{++}$ , 60  $\mu\text{M}$  GSH, and 2 mM PAH (Fig. 3C). Similar to the other two sets of experiments utilizing PAH, the mean rate of inward basolateral flux of  $\text{Hg}^{++}$  in the tubules exposed to PAH was about 65% lower than that in the corresponding tubules not exposed to PAH.

*Effects of glutarate on the basolateral flux and accumulation of  $\text{Hg}^{++}$ .* There was also a statistically significant difference in the mean rate of basolateral uptake of  $\text{Hg}^{++}$  between the group of tubules exposed to  $20 \mu\text{M Hg}^{++}$  and 80  $\mu\text{M}$  Cys and the group of tubules exposed to  $20 \mu\text{M Hg}^{++}$ , 80  $\mu\text{M}$  Cys, and 4 mM glutarate (Fig. 4). The mean rate of inward basolateral flux of  $\text{Hg}^{++}$  in the tubules exposed to glutarate was about 38%

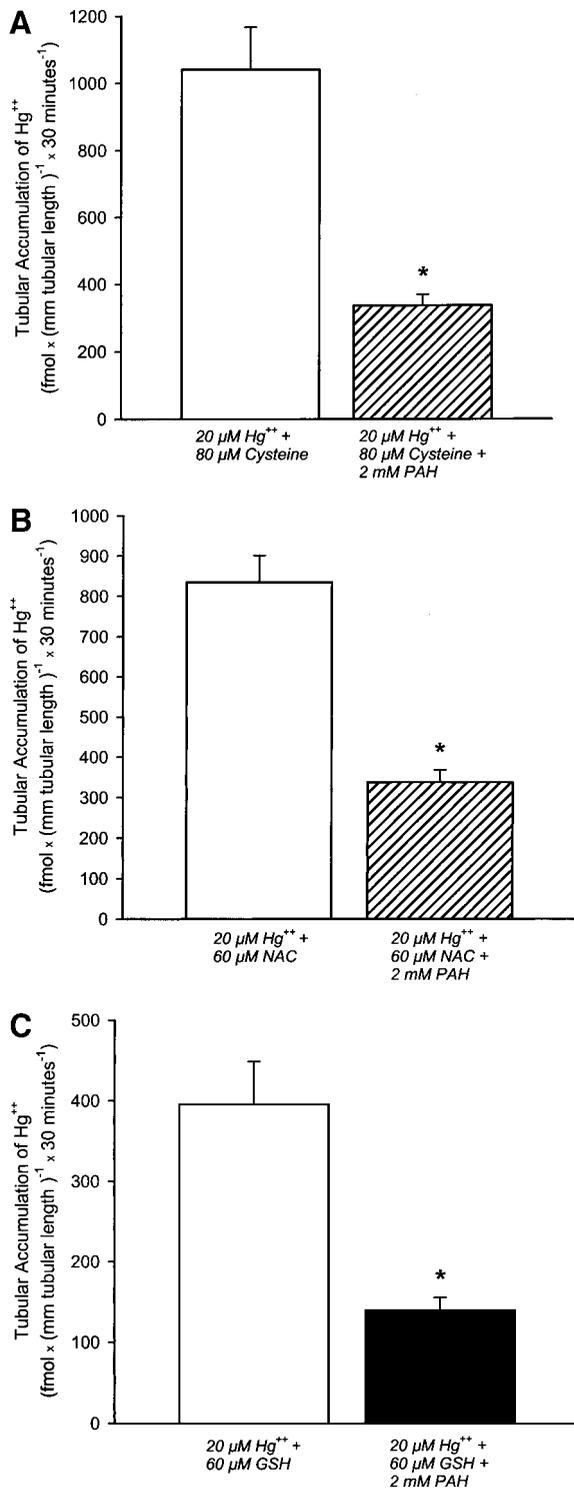
lower than that in the corresponding tubules not exposed to glutarate.

## DISCUSSION

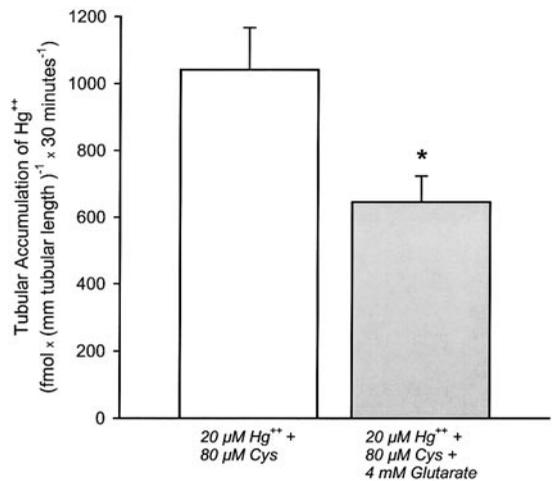
Prior to the present study, mainly indirect, whole animal data have served as the primary support for the hypothesis that basolateral uptake of  $\text{Hg}^{++}$  occurs in proximal tubular epithelial cells *in vivo* and that this transport occurs by a carrier-mediated process (Foulkes, 1974; Zalups, 1995, 1998a,b; Zalups and Barfuss, 1995; 1998a,b), likely involving the extracellular to intracellular movement of mercuric conjugates of specific low-molecular-weight thiols, such as Cys, NAC, homocysteine (homoCys), and/or GSH (Zalups, 1995, 1998a,b; Zalups and Barfuss, 1995, 1998a,b). These *in vivo* data have also served as the only line of evidence indicating that the basolateral uptake of  $\text{Hg}^{++}$  occurs primarily at the site of the PAH-sensitive organic anion transport system (Zalups, 1995, 1998a,b; Zalups and Barfuss, 1995; 1998a,b). Despite all of the data gathered to date, there has been a lack of direct, mechanistic evidence establishing that basolateral uptake of  $\text{Hg}^{++}$  (in the form of mercuric conjugates of biologically relevant low-molecular-weight thiols) occurs along the proximal tubule and that the organic anion transport system is involved.

As a result of the present study, we obtained and provide the most conclusive, direct line of evidence to date (from isolated perfused S2 segments) that carrier-mediated inward basolateral transport of  $\text{Hg}^{++}$  occurs in the epithelial cells lining intact pars recta segments of the proximal tubule. More specifically, the present findings indicate that inward basolateral flux of  $\text{Hg}^{++}$  occurs when the extracellular surface of the basolateral membrane of proximal tubular epithelial cells is exposed to mercuric conjugates Cys, NAC, or GSH, which are, or have been, implicated as being biologically relevant mercuric conjugates involved in the renal proximal tubular uptake of  $\text{Hg}^{++}$  (reviewed in Zalups, 2000). The present findings also show that mercuric conjugates of Cys and NAC are taken up and transported into the proximal tubular epithelial cells at the basolateral membrane more readily than mercuric conjugates of GSH.

Despite their lower rates of uptake, mercuric conjugates of GSH are likely important solutes present in the basolateral and peritubular compartments of proximal tubular segments *in vivo*. To be more specific, in addition to the fact that reduced GSH and Cys are both present in the plasma at a concentration of approximately  $10 \mu\text{M}$  (Lash and Jones, 1985), recent evidence from isolated perfused proximal tubular segments indicates that between 25 and 30% of the GSH synthesized *de novo* in proximal tubular epithelial cells is secreted into the basal and peritubular compartments surrounding the tubule (Parks *et al.*, 1998). Consequently, there is likely a substantial pool of reduced GSH (and Cys) in the peritubular blood (surrounding proximal tubular segments) to compete for mercuric ions bonded to other molecules (such as albumin) to form mercuric



**FIG. 3.** Rate of basolateral accumulation (fmol  $\times$  mm (tubular length) $^{-1}$   $\times$  30 min $^{-1}$ ) of inorganic mercuric ions ( $Hg^{++}$ ) in S2 segments of the rabbit proximal tubule exposed to (A) 20  $\mu$ M  $Hg^{++}$  and 80  $\mu$ M cysteine (Cys), (B) 20  $\mu$ M  $Hg^{++}$  and 60  $\mu$ M *N*-acetylcysteine (NAC), or (C) 20  $\mu$ M  $Hg^{++}$  and 60  $\mu$ M glutathione (GSH), in the presence or absence of 2 mM *p*-aminohipurate (PAH), at their basolateral membrane. All tubular segments were perfused through the lumen with a basic electrolyte solution that did not contain any mercury. Inasmuch as the net rate of flux of  $Hg^{++}$  (fmol  $\times$  mm

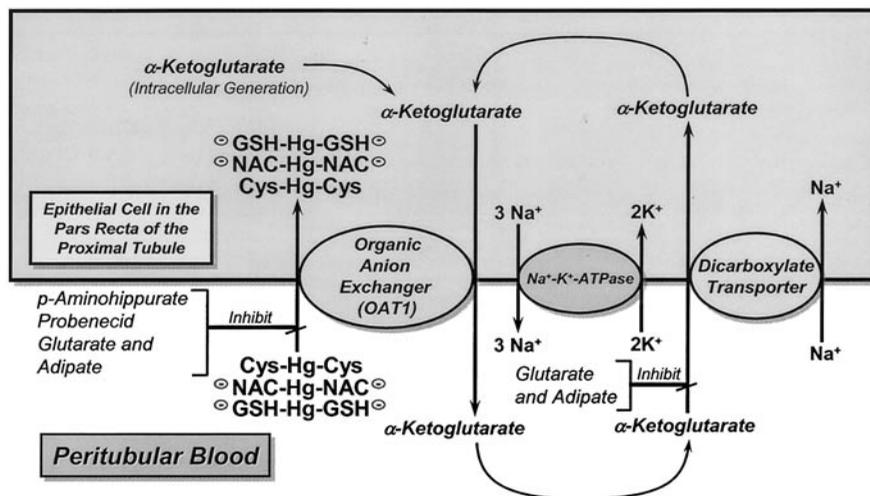


**FIG. 4.** Rate of basolateral accumulation (fmol mm  $\times$  (tubular length) $^{-1}$   $\times$  30 min $^{-1}$ ) of inorganic mercuric ions ( $Hg^{++}$ ) in S2 segments of the rabbit proximal tubule exposed to 20  $\mu$ M  $Hg^{++}$  and 80  $\mu$ M cysteine (Cys), in the presence or absence of 4 mM glutarate, at their basolateral membrane. All tubular segments were perfused through the lumen with a basic electrolyte solution that did not contain any mercury. Inasmuch as the net rate of flux of  $Hg^{++}$  (fmol mm  $\times$  (tubular length) $^{-1}$   $\times$  min $^{-1}$ ) from the bathing solution into the tubular lumen ( $J_{A-BL}$ ) was statistically negligible, the rate of accumulation approximated the rate at which  $Hg^{++}$  was taken up at basolateral membrane. Each value represents the mean  $\pm$  SE for data obtained from six to eight tubular segments. \*Significantly different ( $p < 0.05$ ) from the other mean.

conjugates of GSH (and/or Cys), which can then be transported into the proximal tubular cells at their basolateral membrane.

One of the most significant aspects of the present findings is they provide the most direct evidence to date that the PAH-sensitive organic anion transport system serves as the primary mechanism involved in the inward basolateral transport of  $Hg^{++}$  in pars recta segments of the proximal tubule. Specifically, we demonstrate that, when PAH is present in the basolateral compartment (at a concentration exceeding the  $K_m$  by at least one order of magnitude), the net basolateral uptake of  $Hg^{++}$ , in the form of mercuric conjugates of Cys, NAC, or GSH, is inhibited by 60–70% in S2 proximal tubular segments. These *in vitro* findings also serve as strong support for the conclusion that reductions of the *in vivo* renal uptake and accumulation of  $Hg^{++}$  that are induced in PAH-pretreated rats injected (iv) with mercuric conjugates of Cys, NAC, or GSH are due mainly to inhibition of a basolateral mechanism involved in the uptake of  $Hg^{++}$  in proximal tubular epithelial cells (Zalups, 1995, 1998a,b; Zalups and Barfuss, 1995; Zalups and Minor, 1995). Moreover, and perhaps most importantly, these findings indicate that the reduced proximal tubular uptake

(tubular length) $^{-1}$   $\times$  min $^{-1}$ ) from the bathing solution into the tubular lumen ( $J_{A-BL}$ ) was statistically negligible, the rate of accumulation approximated the rate at which  $Hg^{++}$  was taken up at basolateral membrane. Each value represents the mean  $\pm$  SE for data obtained from six to eight tubular segments. \*Significantly different ( $p < 0.05$ ) from the other mean.



**FIG. 5.** Diagrammatic representation of the putative roles of both the dicarboxylate/organic anion exchanger (OAT1) and the sodium-dependent dicarboxylate transporter in the basolateral uptake of inorganic mercury ( $\text{Hg}^{++}$ ) along the pars recta of the proximal tubule. Based on current evidence on the mechanisms of action of the organic anion transport system, the intracellular concentration of  $\alpha$ -ketoglutarate (resulting from normal metabolic processes and cellular reclamation) creates a chemical gradient facilitating the movement of this dicarboxylate out of the cell at the basolateral membrane by exchanging with organic anions at OAT1 (and perhaps other organic anion transporters) (Dantzer, 1996; Dantzer *et al.*, 1995; Pritchard and Miller, 1993; Roch-Ramel *et al.*, 1992). Once in the basolateral, extracellular compartment, a fraction of the  $\alpha$ -ketoglutarate is taken back up into the proximal tubular epithelial cell across the basolateral membrane via a sym-port involving the cotransport with sodium. This sym-port is driven by the sodium gradient generated by the  $\text{Na}^+$ -K $^+$ -ATPase localized in the basolateral membrane. There are estimates that as much as 50% of the intracellular concentration of  $\alpha$ -ketoglutarate is derived from basolateral reclamation by the proximal tubular epithelial cells through the actions of the sodium-dependent dicarboxylate transporter. According to the data from the present study, inorganic mercury, in the form of mercuric conjugates of cysteine (Cys), *N*-acetylcysteine (NAC), or glutathione (GSH) can enter the epithelial cells of pars recta segments of proximal tubules by one or more mechanisms located in the basolateral membrane. The data obtained with *p*-aminohippurate (PAH) indicate that a significant amount (if not all) of the inorganic mercury that crosses the basolateral membrane is transported into the intracellular compartment through the actions OAT1 (in exchange for intracellular  $\alpha$ -ketoglutarate). The fact that glutarate was able to significantly inhibit the basolateral uptake of mercuric conjugates of cysteine is consistent with the fact showing that the five- and six-carbon dicarboxylates glutarate and adipate compete with  $\alpha$ -ketoglutarate at the site of the dicarboxylate transporter and OAT1. Both glutarate and adipate, but not succinate, can influence the basolateral uptake of mercury by theoretically acting at these two sites (Zalups and Barfuss, 1998a). Evidence indicates that these two dicarboxylic acids can compete for the uptake of substrates transported by the organic anion transporter and other dicarboxylic acids (such as  $\alpha$ -ketoglutarate) transported by the sodium-dicarboxylic acid sym-port system. Succinate, on the other hand, appears to influence the basolateral uptake of mercury by competing only at the site of the dicarboxylate transporter. Preferential uptake of succinate over  $\alpha$ -ketoglutarate would result in a decrease in the intracellular concentration of  $\alpha$ -ketoglutarate, which would decrease the driving force behind the activity of the organic anion transporter.

of  $\text{Hg}^{++}$  induced by PAH *in vivo* is due to inhibition of uptake of specific mercuric conjugates at the site of the organic anion transport system.

Additional evidence indicating that the organic anion transport system is involved in the basolateral uptake of specific mercuric conjugates along the proximal tubule comes from our findings obtained with glutarate. We demonstrate that this dicarboxylate, which is a transportable substrate at both the sodium-dependent dicarboxylate transporter and OAT1, inhibits significantly the basolateral uptake of mercuric conjugates of Cys. This finding is consistent with the *in vivo* findings of Zalups and Barfuss (1998a), which show that pretreatment of rats with glutarate or adipate inhibits significantly the proximal tubular uptake of administered  $\text{Hg}^{++}$  in a dose-dependent manner. The findings obtained when PAH or glutarate were present in the basolateral compartment, therefore, provide strong support for the hypothesis that the organic anion transport system, and more specifically OAT1, is the primary mechanism involved in the basolateral uptake of  $\text{Hg}^{++}$  in pars recta segments

of the proximal tubule. A diagrammatic outline of the role of OAT1 and the sodium-dependent dicarboxylate transporter in the basolateral uptake of mercuric conjugates is presented in Fig. 5.

OAT1 has been shown to serve as the primary inward carrier of various neutral and anionic organic molecules. The gene for OAT1 has been cloned recently from the kidneys of rats (rOAT1; Sekine *et al.*, 1997; Sweet *et al.*, 1997) and humans (hOAT1; Cihlar *et al.*, 1999; Hosoyamada *et al.*, 1999; Lu *et al.*, 1999; Reid *et al.*, 1998) and its expression has been studied in a number of systems. Recent molecular data have confirmed that OAT1 is a multispecific carrier that mediates uptake of structurally dissimilar organic acids and some neutral compounds. In addition to the transport of PAH, OAT1 has been shown to transport certain nonsteroidal antiinflammatory drugs (Apiwattanakul *et al.*, 1999),  $\beta$ -lactam antibiotics (Jariyawat *et al.*, 1999), cyclic nucleotides (Sekine *et al.*, 1997), nucleotide analogs (Cihlar *et al.*, 1999), and mercapturic acids (Pombrio *et al.*, 2001). Preliminary data from a collaboration between

our laboratory and the laboratory of John Pritchard at the NIEHS indicate that when *OAT1* is stably transfected into MDCK cells (which are epithelial cells derived from the distal nephron of the dog that do not express *OAT1*) the cells gain the ability to transport mercuric conjugates of Cys, NAC, or GSH into their cytosolic compartment (Aslamkhan *et al.*, 2002). More importantly, the uptake of these conjugates could be inhibited with probenecid or PAH, indicating that *OAT1* can transport mercuric conjugates of Cys, NAC, or GSH into renal epithelial cells. When these preliminary findings are combined with those of the present study, they provide strong support for the hypothesis that *OAT1* is the primary transporter involved in the basolateral uptake of  $\text{Hg}^{++}$  in proximal tubular epithelial cells *in vivo*.

As alluded to in the introduction, the pars recta (S2 and S3) segments of the proximal tubule are portions of the nephron that are most vulnerable to the nephrotoxic effects of  $\text{Hg}^{++}$  (reviewed by Zalups 2000). Although the precise mechanisms by which  $\text{Hg}^{++}$  induces its toxic effects in these segments are not well defined, the rates of both luminal and basolateral uptake of  $\text{Hg}^{++}$  likely play an important role. Since the basolateral uptake of  $\text{Hg}^{++}$  appears to account for as much as 50–60% of the net amount of  $\text{Hg}^{++}$  that enters and accumulates in the kidneys *in vivo* (Zalups, 1995, 1998a,b; Zalups and Barfuss, 1995; 1998a,b) and, since the greatest activity of the PAH-sensitive organic anion transport system is found in the basolateral membrane of the pars recta segments of the proximal tubule (Pritchard and Miller, 1993; Roch-Ramel *et al.*, 1992), we hypothesize that the basolateral uptake of  $\text{Hg}^{++}$  that is mediated by *OAT1* in these segments plays an important role in the expression of the toxic effects of  $\text{Hg}^{++}$ . Some of the morphological findings from the present study support this hypothesis. To recapitulate, some toxic effects, although very minor, were detected in S2 segments when they were exposed to mercuric conjugates of Cys, NAC, or GSH at their basolateral membrane. However, when either PAH or glutarate was present in the basolateral compartment, there was not only a complete absence of any tubular pathology, but these segments appeared very healthy and pristine morphologically. Therefore, the morphological findings suggest that the activity of the organic anion transport system is linked to the expression of toxicity of  $\text{Hg}^{++}$  when S2 segments are exposed to mercuric conjugates of low-molecular-weight thiols at their basolateral membrane.

In conclusion, the findings from the present study demonstrate clearly that, when mercuric conjugates of Cys, NAC, or GSH are applied directly to the basolateral membrane of S2 segments of the rabbit proximal tubule,  $\text{Hg}^{++}$  is taken up primarily as a mercuric conjugate of the respective thiol at the site of organic anion transport system (specifically *OAT1*).

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