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Mechanisms of Action of 2,3-Dimercaptopropane-1-sulfonate and the Transport, Disposition, and Toxicity of Inorganic Mercury in Isolated Perfused Segments of Rabbit Proximal Tubules

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ABSTRACT

Mechanisms by which the dithiol chelating agent 2,3-dimercaptopropane-1-sulfonate (DMPS) significantly alters the renal tubular transport, accumulation, and toxicity of inorganic mercury were studied in isolated perfused pars recta (S2) segments of proximal tubules of rabbits. Addition of 200 μM DMPS to the bath provided complete protection from the toxic effects of 20 μM inorganic mercury in the lumen. The protection was linked to decreased uptake and accumulation of mercury. Additional data indicated that, when DMPS and inorganic mercury were coperfused through the lumen, very little inorganic mercury was taken up from the lumen. We also obtained data indicating that DMPS is transported by the organic anion transport system and that this transport is linked to the therapeutic effects of DMPS. Interestingly, very little inorganic mercury was taken up and no cellular pathological changes were detected when inorganic mercury and DMPS were added to the bath. We also tested the hypothesis that DMPS can extract cellular mercury while being transported from the bath into the luminal compartment. Our findings showed that, when DMPS was applied to the basolateral membranes of S2 segments after they had been exposed to mercuric conjugates of glutathione of the laminal membrane, the tubular content of mercury was greatly reduced and the rates of disappearance of mercury from the lumen changed from positive values to markedly negative values. We conclude that inorganic mercury is extracted from proximal tubular cells by a transport process involving the movement of DMPS from the bathing compartment to the luminal compartment.

The primary target organ in which inorganic mercury accumulates and expresses toxic effects is the kidney. Experimental evidence indicates that inorganic mercury accumulates primarily along the three segments of the proximal tubule and expresses *in vivo* toxicity mainly in the pars recta of the proximal tubule (Zalups and Lash, 1994). Recent findings from our laboratories indicate that the chelating agent DMPS can act as a rescue agent against the toxic effects of inorganic mercury when it is administered shortly after exposure to mercuric chloride (Zalups *et al.*, 1991a). In addition, it has been shown that this chelating agent can rapidly reduce the renal burden of mercury and increase the urinary excretion of mercury when it is administered after exposure to inorganic mercury (Gabard, 1976; Planas-Bohne, 1981; Hursch *et al.*, 1985; Zalups, 1993; Zalups and Lash, 1994). Despite these important findings, very little is known about the molecular and cellular mechanisms by which DMPS provides protection to proximal tubular epithelial cells from the nephrotoxic effects of mercuric compounds and the manner in which DMPS reduces the renal (proximal) tubular burden of inorganic mercury.

Experimental findings indicate that DMPS is secreted by the probenecid- and PAH-sensitive, organic anion transport system located on the basolateral membrane of proximal tubular epithelial cells (Stewart and Diamond, 1987, 1988; Klotzbach and Diamond, 1988), particularly those lining the pars recta (Roch-Ramel *et al.*, 1992). It is interesting that the primary site along the nephron where DMPS is presumed to be transported corresponds to the site along the nephron where inorganic mercury accumulates avidly and where the toxic effects of inorganic mercury are expressed (Zalups and Lash, 1994). Therefore, it seems logical to hypothesize that there is an intimate link between the site of transport of DMPS and the site of transport and accumulation of inorganic mercury within the kidney. Additional support for this hypothesis comes from recent data implicating the activity of

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ABBREVIATIONS: DMPS, 2,3-dimercaptopropane-1-sulfonate; PAH, *p*-aminohippurate; TCA, trichloroacetic acid; GSH, glutathione; ANOVA, analysis of variance.

the organic anion transport system in the basolateral uptake and transport of inorganic mercury along the proximal tubule (Zalups, 1995; Zalups and Barfuss, 1995, 1998; Zalups and Minor, 1995).

Mercuric ions bind avidly to the vicinal sulfhydryl groups on DMPS. When inorganic mercury binds to DMPS, it forms stable, water-soluble complexes that are excreted readily in the urine (Aposhian, 1983; Ruprecht, 1997). However, all of the mechanisms involved in the increased urinary excretion of mercury after treatment with DMPS are not known. Although several hypotheses have been put forth, it is not clear from the currently available evidence whether DMPS functions primarily as an extracellular chelating agent or whether transport of some forms of DMPS occurs in proximal tubular epithelial cells, with subsequent intracellular binding to inorganic mercury and then extraction of some mercuric conjugate(s) of DMPS into the luminal compartment. It is also not clear whether mercuric conjugates of DMPS are taken up at the luminal and/or basolateral plasma membranes of proximal tubular epithelial cells. Even though there is evidence that DMPS is secreted along the proximal tubule via the organic anion transport system (Stewart and Diamond, 1987, 1988; Klotzbach and Diamond, 1988), it is not certain whether a mercuric conjugate of DMPS can be transported similarly.

Therefore, we evaluated, using isolated perfused pars recta segments of proximal tubules, the following mechanistic questions, in an attempt to better understand the modes of actions of DMPS in the kidney after exposure to inorganic mercury. 1) Are mercuric conjugates of DMPS taken up readily at the luminal and/or basolateral membranes of proximal tubular cells? 2) Is DMPS capable of binding to and extracting inorganic mercury from proximal tubular epithelial cells? 3) Is DMPS taken up at the basolateral membrane by the organic anion transport system, and can its uptake and subsequent therapeutic protective effects in the proximal tubule be prevented by using known inhibitors of the organic anion transport system? The use of the isolated perfused tubule technique afforded us the opportunity to study the pharmacological and therapeutic effects of DMPS on the tubular nephropathy induced by inorganic mercury, in a dynamic *in vitro* system in which intact segments of the nephron were perfused through the lumen under conditions similar to those found *in vivo*. We present significant new data on the mechanisms by which DMPS alters, in a therapeutic manner, the proximal tubular transport, accumulation, and toxicity of inorganic mercury.

Materials and Methods

Animals

Female, specific pathogen-free New Zealand White rabbits (purchased from Myrtle's Rabbit Farm, Thompson Station, TN) were used in the present study. Before experimentation, the rabbits were maintained on regular rabbit chow and given water *ad libitum*. All experiments were conducted according to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Procedure for Obtaining S2 Segments of Proximal Tubules

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). When the rabbit was in a state of deep anesthesia (as determined by the corneal reflex), the abdomen was opened and the kidneys were removed and placed in a cold (4°) aqueous phosphate-sucrose buffer solution. The phosphate-sucrose buffer solution contained 125 mM sucrose, 13.3 mM NaH_2PO_4, and 56 mM Na_2HPO_4, and the pH of the solution was adjusted to 7.4 with 1.0 M NaOH. The kidneys were then quickly sliced into 1–2-mm coronal sections, using a single-edge razor blade. The sections were stored in the same phosphate-sucrose buffer solution. S2 segments of the proximal tubules, 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 8–12 hr. This particular segment of the proximal tubule was chosen for study because it can be readily dissected from rabbit kidney, it makes up the major portion of the pars recta of the proximal tubule (which avidly accumulates inorganic mercury and is involved in the nephropathy induced by this toxic metal), and it is the segment of the nephron in which the greatest activity of the organic anion transport system has been documented (Roch-Ramel *et al.*, 1992).

Method for Perfusing S2 Segments of Proximal Tubules

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, whereas the other set was used to collect the perfused fluid. Every tubule was warmed to 37° for 15 min before the beginning of an experiment. Perfusion rates were maintained, on average, at 7–10 nl/min, with constant hydrostatic pressure. Because of differences in tip diameters of the perfusion pipettes used in the present study, the hydrostatic pressure needed to perfuse S2 segments of proximal tubules at 7–10 nl/min varied between 15 and 50 mm Hg. It should be stressed that most of the drop in pressure occurred at the tip of the perfusion pipette. In addition, each perfused tubule was monitored for any 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 accurately collect 30–50 nl). Bathing fluid surrounding the outside basolateral surface of the perfused tubule was pumped into the bathing chamber at a rate of approximately 0.3 ml/min, continually aspirated, and collected into scintillation vials at 5-min intervals. The perfusion chamber contained 0.3 ml of fluid. For additional details on the methods used to isolated and perfuse segments of proximal tubules of rabbits, refer to the report by Zalups *et al.* (1991b).

Compositions of Perfusing and Bathing Solutions

In all of our experiments, the perfusing and bathing solutions consisted of simple electrolyte solutions. The perfusing solution contained 145 mM Na^+, 140 mM Cl^-, 5 mM K^+, 2.5 mM Ca^{2+}, 1.2 mM Mg^{2+}, 1.2 mM SO_4^{2-}, 2 mM $HPO_4^{2-}/H_2PO_4^-$, 1 mM D-glucose, and 0.5 mM glutamine, and the pH was adjusted to 7.4. To evaluate the cytotoxicity of inorganic mercury, we placed the vital dye FD&C Green 3 (809 Da) in the perfusate at a concentration of 250 nM. The final osmolality was adjusted to 290 mOsmol/kg of water with doubly distilled and deionized water. In all experiments, the primary composition of the bathing solution was the same as that of the perfusing solution. L-[^{3}H]Glucose (50 μCi/ml, 58.8 mCi/mg) was used as a volume marker in all experiments and was added to either the perfusing or bathing solution, depending on the type of experiment being performed. Experimental solutes, such as inorganic mercury (containing $^{203}Hg^{2+}$ at 30 μCi/μg), DMPS, PAH, or glutarate, were added to the perfusing and/or bathing solutions in particular experiments. Refer to Experimental Design for additional details on perfusion conditions.

Collection of Samples

Samples of luminal fluid exiting from the perfused tubular segment (collectate) and bathing solution were collected for each per-

fused tubule, to measure the rates of lumen-to-bath flux or bath-to-lumen flux (femtomoles per minute per millimeter) of inorganic mercury and the volume marker. The time required to fill the constant-volume pipette was used to calculate the volume collection rate (nanoliters per minute).

During the lumen-to-bath flux experiments, the bathing solution was routinely collected and analyzed for the appearance of inorganic mercury and the volume marker (being studied) in the bathing solution. The aspirated bathing solution was collected in 20-ml scintillation vials that were configured into a vacuum trap. Scintillation vials were changed approximately every 5 min during experiments.

Harvesting of Perfused Tubular Segments at the End of the Experiments

To calculate the cellular content of inorganic mercury in the isolated perfused S2 segments of proximal tubules, it was necessary to harvest the perfused tubule at the end of each experiment. The tubular segment was harvested while it was being perfused; it was removed from the bathing solution and placed in 10 μl of 3% (w/v) TCA. The TCA precipitated all of the larger cellular proteins and released the cytosolic contents. After a few minutes, the tubular segment was removed from the TCA solution, placed in a vial with scintillation fluid, and later analyzed (by standard isotopic methods) for the contents of both inorganic mercury and the volume marker L-[^{3}H]glucose. The data obtained for the TCA-soluble and TCA-precipitable fractions permitted us to approximate the cellular content of inorganic mercury.

Experimental Design

Overall design. The present investigation actually consisted of three separate studies. In the first study (study 1), lumen-to-bath flux experiments were carried out under various conditions in which S2 segments were perfused through the lumen with an electrolyte solution containing 20 μM inorganic mercury (some of which was in the form of $^{203}Hg^{2+}$). These experiments were designed to test three main hypotheses. We first tested the hypothesis that DMPS provides protection to S2 segments of proximal tubules when it is presented to the basolateral membranes of these segments while they are being perfused through the lumen with a cytotoxic (20 μM) concentration of inorganic mercury. We then tested the hypothesis that the effects of DMPS are mediated through a mechanism involving the transport of DMPS by the organic anion transporter and that the (postulated) protective effects of DMPS can be reduced or prevented by using compounds known to inhibit the organic anion transport system. The final hypothesis tested in study 1 was that inorganic mercury is not readily taken up at the luminal membrane by S2 segments of proximal tubules when it is in the presence of an excess of DMPS. It was assumed, in this third set of experiments, that all of the mercuric ions were bound to DMPS molecules in thermodynamically stable complexes at the time of perfusion.

In the second study (study 2), the single question of whether mercuric conjugates of DMPS are readily taken up across the basolateral membrane of proximal tubular cells was addressed. In the last study (study 3) of the present investigation, we tested the hypothesis that one mode by which DMPS reduces the renal tubular burden of mercury is by being transported into proximal tubular epithelial cells across the basolateral membrane and then binding to, and subsequently transporting (extracting), cellularly accumulated inorganic mercury out of the cells into the luminal compartment.

Perfusion conditions in study 1. Five separate experiments were performed in study 1. With one set of conditions, lumen-to-bath experiments were performed in which S2 segments were perfused through the lumen with 20 μM inorganic mercury. These experiments served as controls. In another set of experiments, 200 μM DMPS was present in the bathing solution while S2 segments were perfused through the lumen with 20 μM inorganic mercury. These experiments allowed us to evaluate the effects of basolateral application of DMPS on the transport and toxicity of inorganic mercury perfused through the tubular lumen. The rationale for choosing 200 μM DMPS in these experiments relates to the affinity and K_m of organic anions for the organic anion transporter. Because the affinity and K_m of PAH for the organic anion transport system in proximal tubular segments are approximately 100 μM (Roch-Ramel *et al.*, 1992; Dantzler *et al.*, 1995) and because PAH and DMPS appear to have high affinities for the organic anion transporter, we felt that a concentration of DMPS of 200 μM would be appropriate. To evaluate the role of the organic anion transport system on the dispositional and toxicological effects of 200 μM DMPS in the bath, 4.0 mM glutarate or 20 mM PAH was added to the bathing solution. Glutarate and PAH are well established competitive inhibitors of the organic anion transport system, and the doses used effectively block the organic anion transport system. In the last set of experiments in study 1, 20 μM inorganic mercury and 80 μM DMPS were coperfused through the lumen. As alluded to above, we chose to use 80 μM DMPS in these experiments to ensure that all of the mercuric ions in solution would be incorporated into some form of complex with DMPS before perfusion of tubular segments.

Perfusion conditions in study 2. In the control experiments of this study, bath-to-lumen experiments were carried out with 20 μM inorganic mercury in the bathing solution. To examine whether basolateral uptake of inorganic mercury can occur when the mercury is in the form of a mercuric conjugate of DMPS, bath-to-lumen experiments were carried out with S2 segments that were perfused through the lumen with normal artificial perfusion medium and were simultaneously bathed with 20 μM inorganic mercury plus 200 μM DMPS.

Perfusion conditions in study 3. Three separate sets of experiments were carried out in study 3. S2 segments of proximal tubules were perfused through the lumen with 20 μM inorganic mercury and 80 μM GSH. The presence of 80 μM GSH ensured the formation of linear II coordinate covalent bonds between two molecules of GSH and each mercuric ion (Rabenstein, 1989). In recent studies, mercuric conjugates of GSH have been implicated as potential transport forms of inorganic mercury. Moreover, we previously showed that perfusion with mercuric conjugates of GSH provides protection to S2 segments from the toxic effects of inorganic mercury, while allowing inorganic mercury to be transported into the proximal tubular epithelial cells (Zalups *et al.*, 1991b). Each tubular segment was perfused through the lumen for three 5-min collection periods. At the end of the initial 15 min of perfusion, 200 μM DMPS was added to the bathing solution. Three additional 5-min collections were then carried out. Lumen-to-bath transport data were obtained during all six collection periods, and each tubule was also evaluated for cellular toxicity. At the end of the last collection period, the tubular content and distribution of mercury were determined. These experiments allowed us to test the hypothesis that DMPS is rapidly transported into proximal tubular epithelial cells across the basolateral membrane and then readily transports (extracts) accumulated inorganic mercury from proximal tubular epithelial cells into the lumen of the tubule.

Two additional control experiments were carried out for study 3. Lumen-to-bath transport of mercury in S2 segments of proximal tubules was evaluated during three or six 5-min collection periods (in two separate groups of tubules). These two groups of tubules were perfused through the lumen with 20 μM inorganic mercury and 80 μM GSH while DMPS was absent from the bath. At the end of each of these perfusion experiments, the tubular content and distribution of mercury were determined.

Assessment of Cellular and Tubular Pathological Changes

During each experiment, the perfused tubule was observed microscopically during the entire perfusion process, to detect any pathological changes. Typical pathological changes detected in S2 segments of proximal tubules exposed to inorganic mercury included cellular swelling, cytoplasmic vacuolization, shedding of the brush-

border membrane, blebbing of the apical cytoplasm, and uptake of the vital dye FD&C Green 3.

Calculations

Lumen-to-bath flux. Transport of inorganic mercury in lumen-to-bath flux experiments was evaluated by measuring the rate at which radioactive mercuric ions ($^{203}Hg^{2+}$) disappeared from the luminal fluid (disappearance flux rate) (J_D) and the rate at which they appeared in the bathing solution (appearance flux rate) (J_A). The disappearance flux rate for inorganic mercury (femtomoles per minute per millimeter) was calculated with eq. 1,

$$J_D = [([Hg^{2+}]_P \times V_P) - ([Hg^{2+}]_c \times V_C)] \div L \quad (1)$$

where $[Hg^{2+}]_P$ and $[Hg^{2+}]_C$ are the concentrations (femtomoles per nanoliter) of inorganic mercury in the perfusate and collectate, respectively. $[Hg^{2+}]_P$ and $[Hg^{2+}]_C$ were determined from the specific activity (dpm per femtomole) of $^{203}Hg^{2+}$. L is the length (millimeters) of the perfused tubular segment. V_C is the volume collection rate (nanoliters per minute), which was calculated from the time required to fill the constant-volume pipette. V_P is the rate at which fluid was being perfused through the tubular segment (perfusion rate) (nanoliters per minute) and was calculated with eq. 2,

$$V_P = ([VM]_C \div [VM]_P) \times V_C \quad (2)$$

where $[VM]_C$ and $[VM]_P$ are the concentrations of the volume marker (L-[^{3}H]glucose) in the collectate and perfusate, respectively. The concentration of the volume marker in the perfusate or collectate is expressed as dpm per nanoliter, where dpm is the dpm value for the radioisotope being used. The appearance flux rate (J_A) (femtomoles per minute per millimeter) for inorganic mercury was calculated with eq. 3,

$$J_A = [dpm_{Hg} \div (SA_{Hg} \times T)] \div L \quad (3)$$

where dpm_{Hg} represents the amount (dpm) of $^{203}Hg^{2+}$ that appeared in the bathing solution in time T (minutes). SA_{Hg} represents the specific activity (dpm per femtomole) of the $^{203}Hg^{2+}$ used, and L is the length (millimeters) of the tubular segment.

Bath-to-lumen flux. In bath-to-lumen flux experiments, we determined the rate at which inorganic mercury appeared in the luminal fluid from the bathing fluid. This appearance flux (J_{BL}) (femtomoles per minute per millimeter) was calculated by measuring the rate of appearance of the solute in the lumen when the solute was placed in the bathing solution only. Eq. 4 was used for this calculation,

$$J_{BL} = [dpm_{Hg} \div (SA_{Hg} \times T)] \div L \quad (4)$$

where dpm_{Hg} is the amount (dpm) of $^{203}Hg^{2+}$ that appeared in the luminal fluid, SA_{Hg} is the specific activity (dpm per femtomole) of the $^{203}Hg^{2+}$ used, T is the time (minutes) required to collect the sample, and L is the length (millimeters) of the tubular segment.

Cellular content of solute (Hg^{2+}). The content of inorganic mercury (Hg_{Tubule}) in perfused tubular segments was calculated using eq. 5,

$$Hg_{Tubule} = (dpm_{Hg} \div SA_{Hg}) \div L \quad (5)$$

where dpm_{Hg} is the amount (dpm) of $^{203}Hg^{2+}$ in the perfused tubular segment (both TCA-soluble and TCA-insoluble fractions), SA_{Hg} is the specific activity (dpm per femtomole) of $^{203}Hg^{2+}$ taken from standards, and L is the length (millimeters) of the perfused tubular segment.

Leak. Because the epithelial cells in various segments of the nephron permit the intercellular passage (through junctional complexes) of certain solutes to varying degrees, it is important to determine the magnitude of the intercellular leak of a solute and to factor in that leak in calculations of the rates of transport of a solute in a perfused tubular segment. In lumen-to-bath experiments, if the appearance of the volume marker in the bathing solution is greater than its normal rate of appearance, it can be assumed that the perfused tubular segment has been structurally compromised. In intact tubular segments, the intercellular leak of fluid is very low (<0.1 nl/min).

The rate of lumen-to-bath or bath-to-lumen leak of fluid (L_{VM}) (nanoliters per minute) in tubular segments was measured as the rate of appearance of the volume marker in the bathing or luminal fluid, respectively. This was calculated using eq. 6,

$$L_{VM} = dpm_{VM} \div ([VM] \div T) \quad (6)$$

where dpm_{VM} is the amount (dpm) of the radioisotope of the volume marker that appears in the bathing or luminal solution, [VM] is the concentration of the volume marker in the luminal or bathing fluid, expressed as the radioactivity of the volume marker per unit of liquid volume (dpm per nanoliter), and T is the time (minutes) required to collect the sample of bathing or luminal fluid.

Statistical Analysis

Data for each parameter assessed in study 1 were first analyzed with one-way 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 t test was used in study 2 to statistically evaluate the differences between corresponding pairs of means for experimental and control data. In study 3, a one-way repeated-measures ANOVA was used to assess differences among corresponding sets of means. When significant F values were obtained with the ANOVA, Tukey's multiple-comparison procedure was used to detect significant differences among the means. The level of significance for all statistical analyses performed was chosen *a priori* to be $p < 0.05$.

Results

Lumen-to-Bath Experiments of Study 1

Condition 1 (20 μM Hg^{2+} in the lumen) (control tubules). *Transport and Dispositional Data*. Perfusion conditions and additional data for the experiments in study 1 are presented in Table 1. When S2 segments of proximal tubules were perfused through the lumen with 20 μM inorganic mercury in a simple electrolyte solution, there was rapid uptake of mercuric ions by the proximal tubular cells. The rate at which inorganic mercury disappeared from the luminal compartment (J_D) over several collection periods averaged approximately 114 fmol/min/mm (Fig. 1). Only a small portion of the disappearance flux represents intercellular movement of mercury, because the leak of the volume marker was relatively low (compared with the disappearance flux rate), especially during the first collection period. The disappearance flux rate was so large in these experiments that approximately 80% of the inorganic mercury perfused through the lumen was extracted from the luminal fluid (Fig. 2). In addition, by the end of each of these perfusion experiments (approximately 30 min), approximately 1270 fmol of inorganic mercury was present in each 1 mm of perfused tubule (Fig. 3). Most of the inorganic mercury in the tubules at the end of each experiment was associated with the TCA-insoluble fraction of the tubules (Table 1).

Toxicity Data. Within 5 min of the initiation of perfusion, tubular epithelial cells at the end of the tubule attached to the perfusion pipette began to swell, undergo blebbing, and take up the vital dye FD&C Green 3. By the end of the initial 15 min of perfusion, cellular blebbing and dye uptake had progressed along the tubule. At the completion of 30–40 min

of perfusion, approximately 500 μm (approximately one half of the length of the perfused segment) of the proximal end of the perfused segment displayed significant pathological changes, including the uptake of the vital dye. Leak of the volume marker L-[^{3}H]glucose was elevated throughout the perfusion process (Table 1). Intercellular leak of the volume marker was especially elevated during the last collection period, which was generally the last 5–10 min of a 30–40-min perfusion experiment.

Condition 2 (20 μM Hg^{2+} in the lumen and 200 μM DMPS in the bath). *Transport and Dispositional Data.* In experiments in which 200 μM DMPS was added to the solution bathing the tubules being perfused through the lumen with 20 μM inorganic mercury, the disappearance flux rate for inorganic mercury was essentially 0 (Fig. 1). Moreover, the total amount of inorganic mercury that accumulated in the perfused S2 segments during the course of the experiments was very small (<30 fmol/mm of tubule) (Fig. 3). Furthermore, the mean luminal concentration of inorganic mercury averaged approximately 20 μM during all collection periods, indicating that no significant amounts of inorganic mercury had disappeared from the luminal compartment during the perfusion process. This was particularly evident when the concentration of mercury in the collectate was determined (Fig. 2).

Toxicity Data. When S2 segments were bathed with an electrolyte solution containing 200 μM DMPS while they were being perfused through the lumen with 20 μM inorganic mercury, they appeared normal, without any signs of pathological changes, for the entire 30–40 min of perfusion. No evidence of cellular swelling, blebbing, vacuolization, or uptake of the vital dye was detected at any time in any of the tubules during the perfusion process. Leak of the volume marker was very low, which served as evidence of healthy tubules (Table 1).

Condition 3 (20 μM Hg^{2+} in the lumen and 200 μM DMPS plus 4 mM glutarate or 20 μM PAH in the bath). *Transport and Dispositional Data.* To test whether the effects of DMPS (when present in the bathing solution) on the tubular transport and disposition of inorganic mercury in S2 segments of proximal tubules were dependent on the activity of the organic anion transport system, we added 4.0 mM glutarate or 20 mM PAH to the bathing solution. These compounds are established competitive inhibitors of the activity of the organic anion transporter. When 4.0 mM glutarate or 20 mM PAH was present with 200 μM DMPS in the bathing

TABLE 1

Perfusion data for study 1

Values represent the mean ± standard error. Refer to Materials and Methods for details of the experimental design.

Perfusion conditions		Number of tubules studied	Perfusion rate	Mean luminal Hg^{2+} concentration	Content of Hg^{2+} in TCA-soluble tubule fraction	Content of Hg^{2+} in TCA-insoluble tubule fraction	Leak
Present in luminal fluid	Present in bathing fluid						
			nl/min	*μM*	*fmol/mm*	*fmol/mm*	*nl/min*
20 μM Hg^{2+}	None	4	7.37 ± 0.51	11.7 ± 0.32	107 ± 55.8	1028 ± 290	0.31 ± 0.07
20 μM Hg^{2+}	200 μM DMPS	4	10.7 ± 1.23	20.0 ± 0.07	12.9 ± 1.21	4.10 ± 0.46[a]	0.13 ± 0.03[a]
20 μM Hg^{2+}	200 μM DMPS + 4.0 mM glutarate	5	9.16 ± 0.93	11.7 ± 0.09	21.8 ± 9.84	1262 ± 201	0.50 ± 0.09
20 μM Hg^{2+}	200 μM DMPS + 20 mM PAH	4	7.38 ± 0.36	12.6 ± 0.05	29.3 ± 5.05	1046 ± 90.0	0.23 ± 0.01
20 μM Hg^{2+} + 80 μM DMPS	None	5	7.20 ± 0.58	19.5 ± 0.23	−2.16 ± 0.69	88.9 ± 7.80[a]	0.21 ± 0.01

[a] Significantly different ($p < 0.05$) from all of the other corresponding means.

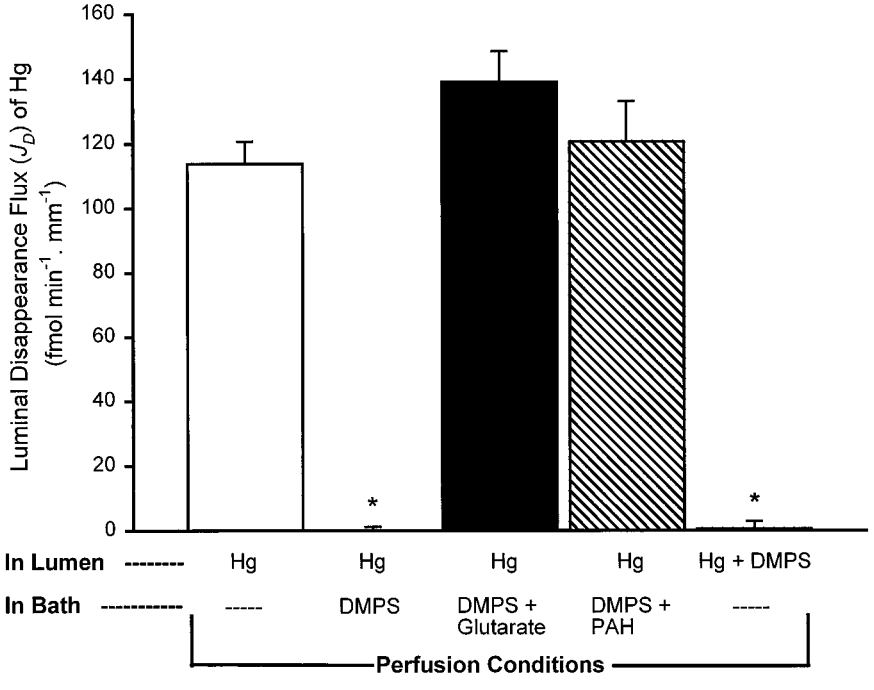

Fig. 1. Rate of disappearance of inorganic mercury from the luminal compartment of isolated perfused S2 segments of proximal tubules of rabbits. Each lumen-to-bath perfusion experiment was initiated after a 15-min warm-up period and was terminated after three 5-min collection periods. The conditions under which each experiment was carried out are outlined below the *x*-axis. *First bar* (from the *left*), data obtained from S2 segments perfused through the lumen with 20 μM inorganic mercury; *second bar*, data obtained from S2 segments perfused through the lumen with 20 μM inorganic mercury while being bathed with 200 μM DMPS; *third bar*, data obtained from S2 segments perfused through the lumen with 20 μM inorganic mercury while being bathed with 200 μM DMPS and 4.0 mM glutarate; *fourth bar*, data obtained from S2 segments perfused through the lumen with 20 μM inorganic mercury while being bathed with 200 μM DMPS and 20.0 mM PAH; *fifth bar*, data obtained from S2 segments perfused through the lumen with both 20 μM inorganic mercury and 80 μM DMPS. *Values*, mean ± standard error obtained from four or five perfused tubules. *, Significantly different ($p < 0.05$) from the means for tubules perfused through the lumen with 20 μM inorganic mercury, perfused through the lumen with 20 μM inorganic mercury while being bathed with 200 μM DMPS and 4.0 mM glutarate, or perfused through the lumen with 20 μM inorganic mercury while being bathed with 200 μM DMPS and 20.0 mM PAH.

solution surrounding the S2 segments being perfused through the lumen with 20 μM inorganic mercury, the disappearance flux rates for inorganic mercury were not significantly different from those detected in control tubules perfused under conditions in which 20 μM inorganic mercury was perfused through the lumen and no DMPS, PAH, or glutarate was present in the bath (Fig. 1). The tubular content of mercury under conditions in which glutarate or PAH was present in the bath was also not significantly different from the tubular content measured in control tubules perfused with 20 μM inorganic mercury.

Toxicity Data. By the end of the first 10–15 min of perfusion, cellular swelling and blebbing and uptake of the vital dye occurred at the perfusion end of S2 segments perfused through the lumen with 20 μM inorganic mercury and bathed with 200 μM DMPS plus 4.0 mM glutarate or 20 mM PAH. After the initial 15 min of perfusion, cellular blebbing and dye uptake progressed further along the tubule. After the initial 15 min, the perfusion pressure needed to be increased. At the completion of 30–40 min of perfusion, approximately 500 μm (approximately one half of the length of the perfused segment) of the proximal end of the perfused segment displayed significant pathological changes, including the uptake of the vital dye. Leak of the volume marker was very high throughout the perfusion process. In general, the pathologi-

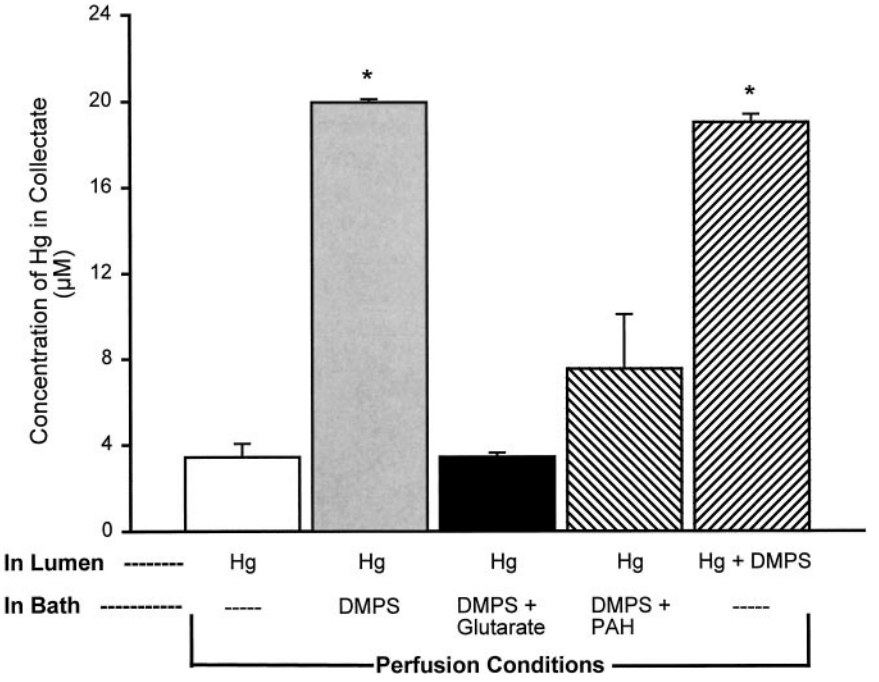

Fig. 2. Concentrations of inorganic mercury in the collectates from isolated perfused S2 segments of proximal tubules of rabbits. Each lumen-to-bath perfusion experiment was initiated after a 15-min warm-up period and was terminated after three 5-min collection periods. Details of the conditions under which each experiment was carried out are provided in the legend to Fig. 1. *Values*, mean ± standard error obtained from four or five perfused tubules. *, Significantly different ($p < 0.05$) from the means for the groups of tubules perfused through the lumen with 20 μM inorganic mercury, perfused through the lumen with 20 μM inorganic mercury while being bathed with 200 μM DMPS and 4.0 mM glutarate, or perfused through the lumen with 20 μM inorganic mercury while being bathed with 200 μM DMPS and 20.0 mM PAH.

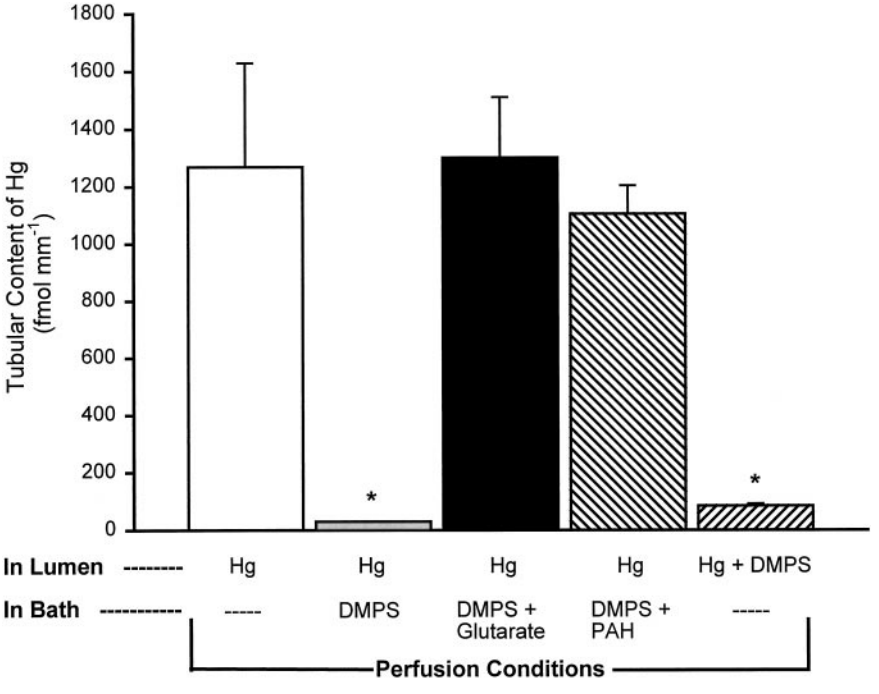

Fig. 3. Content of inorganic mercury in isolated perfused S2 segments of proximal tubules of rabbits. Each lumen-to-bath perfusion experiment was initiated after a 15-min warm-up period and was terminated after three 5-min collection periods. Details of the conditions under which each experiment was carried out are provided in the legend to Fig. 1. *Values*, mean ± standard error obtained from four or five perfused tubules. *, Significantly different ($p < 0.05$) from the means for the groups of tubules perfused through the lumen with 20 μM inorganic mercury, perfused through the lumen with 20 μM inorganic mercury while being bathed with 200 μM DMPS and 4.0 mM glutarate, or perfused through the lumen with 20 μM inorganic mercury while being bathed with 200 μM DMPS and 20.0 mM PAH.

cal features were similar to those seen in tubules perfused with inorganic mercury alone.

Condition 4 (20 μM Hg^{2+} plus 80 μM DMPS in the lumen). *Transport and Dispositional Data.* In the final experiment of study 1, we examined the effect of coperfusing 20 μM inorganic mercury and 80 μM DMPS through the lumen, with no DMPS or inhibitor of the organic anion transporter in the bath. In these experiments, the rates of disappearance of inorganic mercury from the luminal fluid were essentially 0 (Fig. 1) and the net accumulation of mercury in each experiment was, on average, a very low 84 fmol/mm of tubule (Fig. 3). In addition, the mean luminal concentration of inorganic mercury was 19.7 μM during the three collection periods of each experiment (Table 1). This value, with the concentration of mercury in the collectate, indicates that very little or no inorganic mercury disappeared from the luminal compartment during each experiment (Fig. 3).

Toxicity Data. S2 segments appeared normal throughout 30–40 min of perfusion, except for very slight swelling near the perfusion pipette in a few tubules. Leak of the vital dye was also very low, as in normal healthy tubules not perfused with mercury.

Bath-to-Lumen Experiments of Study 2

Condition 1 (20 μM Hg^{2+} in the bath) (control tubules). *Transport and Dispositional Data.* When 20 μM inorganic mercury was applied to the basolateral surfaces of S2 segments of proximal tubules, the rates of uptake and accumulation of mercury were very rapid, as displayed in Fig. 4. Additional perfusion data for study 2 are presented in Table 2.

Toxicity Data. Severe pathological changes, including cellular swelling, blebbing, and necrosis, occurred within the first 5 min of each experiment when the basolateral surfaces of S2 segments were exposed to 20 μM inorganic mercury. Pathological changes occurred along the entire length of each perfused segment.

Condition 2 (20 μM Hg^{2+} plus 200 μM DMPS in the bath). *Transport and Dispositional Data.* Very little uptake of inorganic mercury occurred in S2 segments when their basolateral surfaces were exposed to 20 μM inorganic mercury plus 200 μM DMPS (Fig. 4). Moreover, there was negligible accumulation of inorganic mercury in these tubules, especially compared with the level of accumulation in the control tubules.

Toxicity Data. No cellular pathological features were detected in any experiment in which the basolateral surfaces of S2 segments of proximal tubules were exposed to 20 μM inorganic mercury plus 200 μM DMPS.

Extraction Experiments of Study 3

Transport and dispositional data. During the first three 5-min collection periods, the luminal disappearance flux rate for inorganic mercury in the S2 segments perfused through the lumen with 20 μM inorganic mercury plus 80 μM GSH was, on average, between 44 and 29 fmol/min/mm (Fig. 5). However, after the addition of 200 μM DMPS to the bathing solution, the disappearance flux rate decreased almost immediately to approximately −36.0 fmol/min/mm. During the next two 5-min collection periods, the disappearance flux rate was, on average, between −48 and −44 fmol/min/mm.

The concentration of inorganic mercury in the collectate during the first three collection periods ranged from approximately 10 to 12 μM (Fig. 6), indicating that almost 50% of the mercury in the perfusate was being taken up by the tubular epithelial cells. Interestingly, the concentration of inorganic mercury in the collectate during the last three collection periods was slightly more than 20 μM.

The tubular content of mercury in the set of control tubules perfused through the lumen with 20 μM inorganic mercury plus 80 μM GSH (with no DMPS in the bath) was >400

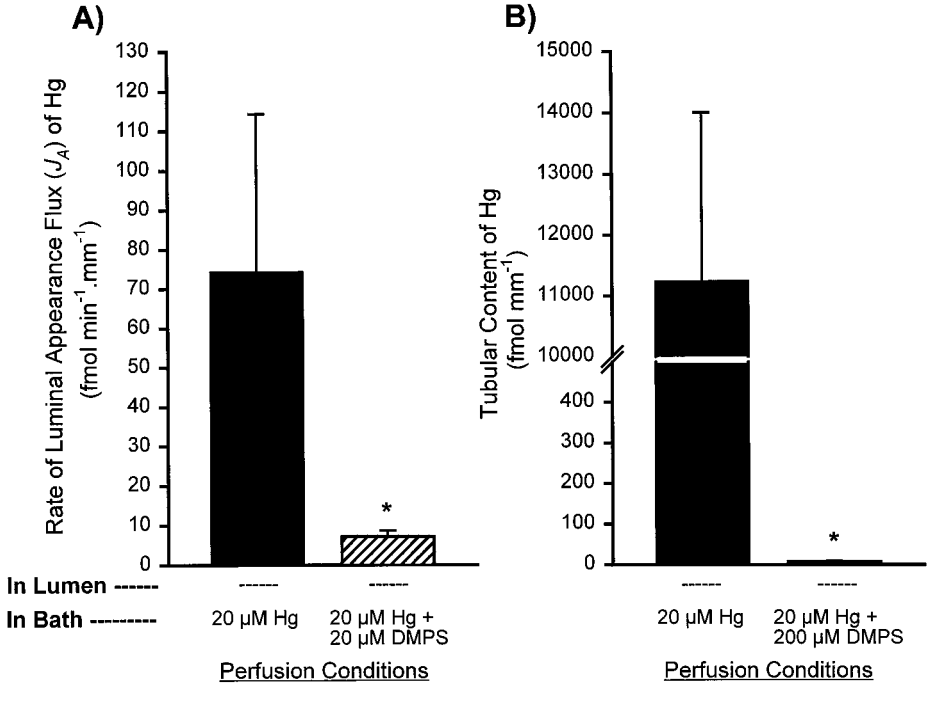

Fig. 4. Luminal appearance flux rate (J_A) for inorganic mercury (A) and tubular contents of inorganic mercury (B) in isolated perfused S2 segments of proximal tubules of rabbits. Each bath-to-lumen perfusion experiment was terminated after three 5-min collection periods. The conditions under which each experiment was carried out are noted below the x-axis. *First bar* (from the *left*), data obtained from perfused S2 segments bathed with 20 μM inorganic mercury; *second bar*, data obtained from perfused S2 segments bathed with 20 μM inorganic mercury and 200 μM DMPS. Each value represents the mean ± standard error obtained from three perfused tubules. *, Significantly different ($p < 0.05$) from the mean for tubules bathed with 20 μM inorganic mercury.

fmol/mm by the end of the third 5-min collection period (Fig. 7). In contrast, the average content of mercury in the experimental tubules (exposed to DMPS on their basolateral surfaces during the last three 5-min collection periods) was slightly more than 100 fmol/mm by the end of the sixth 5-min collection period (Fig. 7). Moreover, the tubular content of inorganic mercury in the control tubules perfused through the lumen with 20 μM inorganic mercury plus 80 μM GSH (with no DMPS in the bath) for six collection periods was, on average, 300 fmol/mm (Table 3). Additional dispositional

TABLE 2

Perfusion data for study 2

Values represent the mean ± standard error obtained from isolated perfused S2 segments of proximal tubules. Refer to Materials and Methods for details of the experimental design.

Perfusion conditions		Number of tubules studied	Mean luminal Hg^{2+} concentration	Content of Hg^{2+} in TCA-soluble tubule fraction	Content of Hg^{2+} in TCA-insoluble tubule fraction	Leak
Present in luminal fluid	Present in bathing fluid					
			μM	*fmol/mm*	*fmol/mm*	*nl/min*
None	20 μM Hg^{2+}	3	0.24 ± 0.20	1.00 ± 0.51	6845 ± 1767	9.03 ± 5.10
None	20 μM Hg^{2+} + 200 μM DMPS	3	0.46 ± 0.12	3.32 ± 3.98	30.7 ± 16.1[a]	0.33 ± 0.06[a]

[a] Significantly different ($p < 0.05$) from all of the other corresponding means.

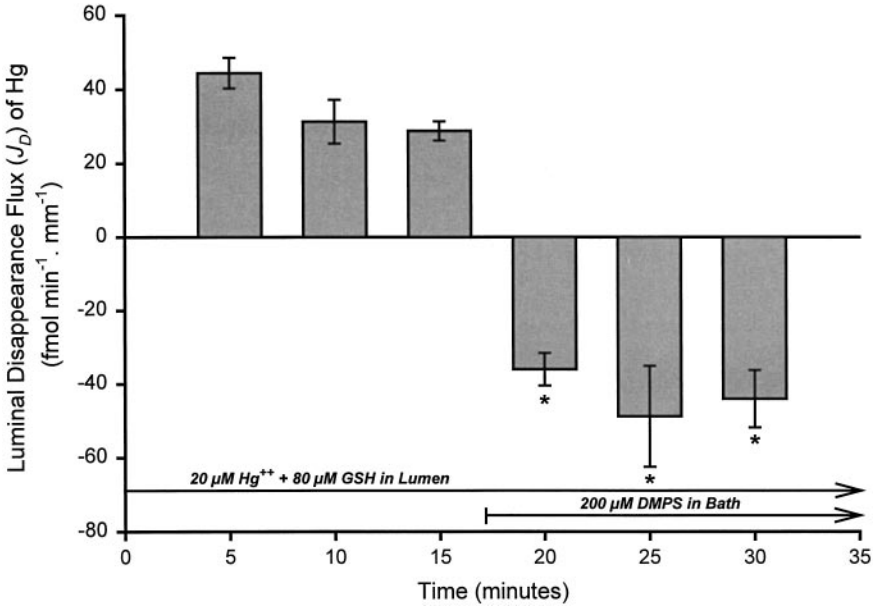

Fig. 5. Rate of disappearance of inorganic mercury from the luminal compartment of isolated perfused S2 segments of proximal tubules of rabbits. All S2 segments in this experiment were perfused through the lumen with 20 μM inorganic mercury and 80 μM GSH for six 5-min collection periods. During the last three 5-min collection periods, 200 μM DMPS was added to the solution bathing the tubules. *Values*, mean ± standard error obtained from six perfused tubules. *, Significantly different ($p < 0.05$) from the means obtained (from the same tubules) during the initial three 5-min collection periods.

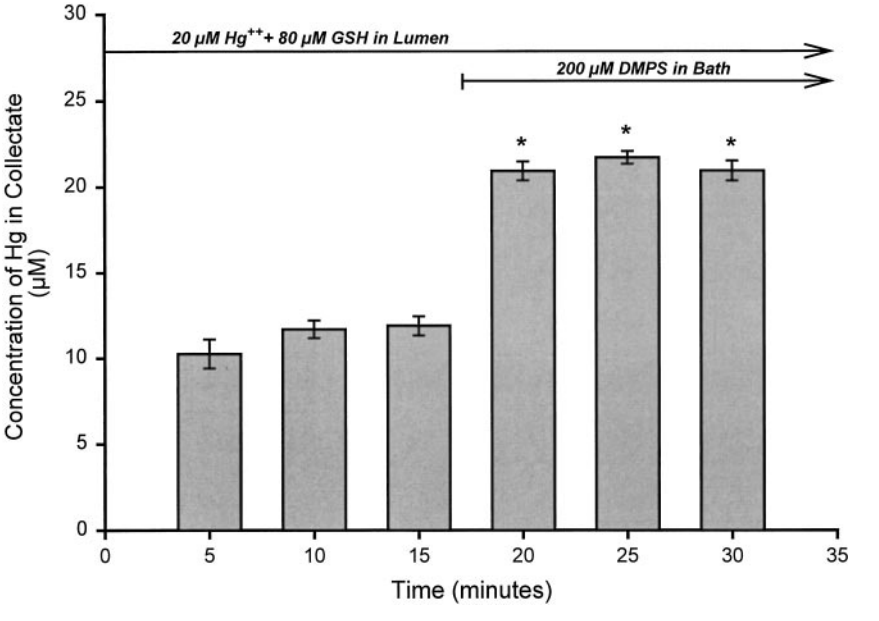

Fig. 6. Concentration of inorganic mercury in the collectate from isolated perfused S2 segments of proximal tubules of rabbits. All S2 segments in this experiment were perfused through the lumen with 20 μM inorganic mercury and 80 μM GSH for six 5-min collection periods. During the last three 5-min collection periods, 200 μM DMPS was added to the solution bathing the tubules. *Values*, mean ± standard error obtained from six perfused tubules. *, Significantly different ($p < 0.05$) from the means obtained (from the same tubules) during the initial three 5-min collection periods.

data, such as the intercellular leak of the volume marker and the cellular distribution of accumulated mercury, are presented in Table 3.

Toxicity data. No significant cellular pathological changes were detected in any of the experiments carried out in study 3.

Oxidation of DMPS at 25°

A separate experiment was carried out to determine the degree of oxidation of DMPS in the bathing solutions used in the present study. The solution used to bathe the perfused S2 segments of proximal tubules was maintained at 25° before being pumped into the bathing chamber. The time between preparation of the bathing solution containing 200 μM DMPS and pumping of the solution into the heated bathing chamber was no more than 5 hr. We felt that it was important to estimate the maximal level of oxidation of the sulfhydryl groups on DMPS that occurred near the end of a sequence of several experiments (generally no more than 5 hr). Using the method of Ellman (1959), we measured the concentration of reduced sulfhydryl groups in the solution used to bathe the tubules. Our findings indicated that approximately 19.8% of the sulfhydryl groups on molecules of DMPS in solution were oxidized by 5 hr after placement of DMPS into aqueous solution (Fig. 8). However, >92% of the sulfhydryl groups in the solution containing DMPS were oxidized after 21 hr. Therefore, our findings indicate that, in the majority of experiments, >80% of the sulfhydryl groups in the bathing solution containing DMPS were in the reduced form. In the very first experiment of each day of perfusion, the amount the sulfhydryl groups in solution that were in the reduced form most likely exceeded 90%.

Discussion

Our findings confirm that, when 20 μM inorganic mercury is perfused through the lumen of pars recta segments of proximal tubules of rabbits, inorganic mercury is taken up and accumulated rapidly by the tubular epithelial cells (Zalups *et al.*, 1991). In addition, the findings confirm that the tubular epithelial cells undergo rapidly progressing pathological changes leading to cellular necrosis, which begins at the end of the tubule attached to the perfusion pipette and progresses down toward the collection pipette. Interestingly, when 200 μM DMPS is added to the solution bathing the perfused tubule, complete protection from the toxic effects of

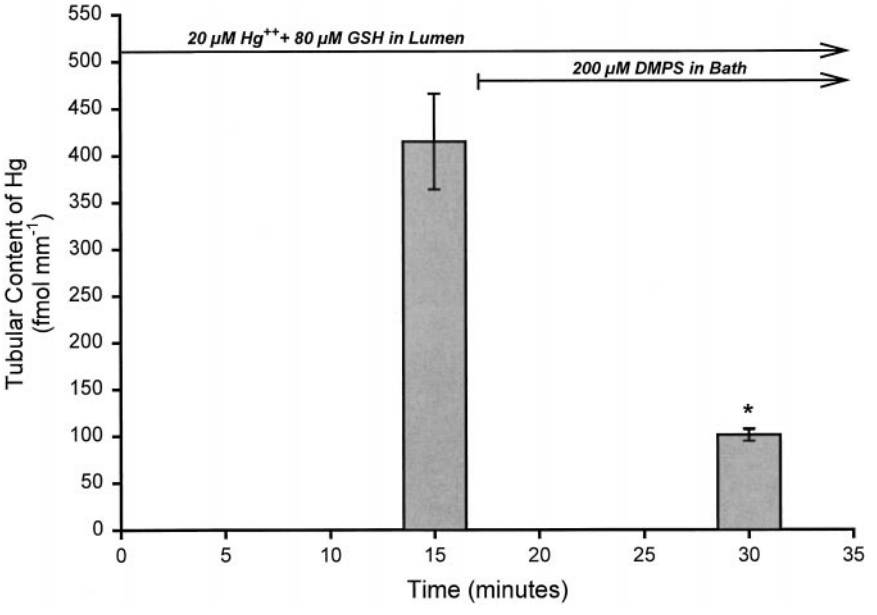

Fig. 7. Content of inorganic mercury in isolated perfused S2 segments of proximal tubules of rabbits. In one set of three control tubules, the tubular content of inorganic mercury was determined after three 5-min collection periods during which 20 μM inorganic mercury and 80 μM GSH were perfused through the lumen. In six experimental tubules, the tubular content of inorganic mercury was determined after six 5-min collection periods. During all six collection periods, the tubules were perfused through the lumen with 20 μM inorganic mercury and 80 μM GSH. However, during the last three collection periods, 200 μM DMPS was added to the solution used to bathe the perfused tubules. *Values*, mean ± standard error. *, Significantly different ($p < 0.05$) from the mean for tubules perfused through the lumen with 20 μM inorganic mercury and 80 μM GSH for three 5-min collection periods.

TABLE 3

Perfusion data for study 3

Values represent the mean ± standard error obtained from isolated perfused S2 segments of proximal tubules. Refer to Materials and Methods for details of the experimental design. In all cases [except as marked ([b]) below], 20 μM Hg^{2+} and 80 μM GSH were present in the luminal fluid.

Perfusion periods for each tubule	Present in bathing fluid	Number of tubules studied	Perfusion rate	Mean luminal Hg^{2+} concentration	Content of Hg^{2+} in TCA-soluble tubule fraction	Content of Hg^{2+} in TCA-insoluble tubule fraction	Leak
			nl/min	*μM*	*fmol/mm*	*fmol/mm*	*nl/min*
1st 15 min	None	6	6.42 ± 0.52	15.6 ± 0.58			0.21 ± 0.06
2nd 15 min	200 μM DMPS		6.31 ± 0.71	20.6 ± 0.40[a]	20.1 ± 9.32	74.9 ± 6.51	0.22 ± 0.06
1st 15 min	None	3	10.7 ± 1.23	17.8 ± 0.22	20.0 ± 4.74	396 ± 54.6	0.09 ± 0.01
2nd 15 min[b]	NA		NA	NA	NA	NA	NA
1st 15 min	None	2	7.62 ± 0.10	17.5 ± 0.64			0.10 ± 0.00
2nd 15 min	None		7.18 ± 0.19	17.0 ± 0.64	30.0 ± 8.45	271 ± 51.7[c]	0.11 ± 0.04

[a] Significantly different ($p < 0.05$) from the corresponding mean obtained during the first 15 min of perfusion.
[b] NA, not applicable, because tubules were not perfused for a second period of 15 min.
[c] Significantly different ($p < 0.05$) from the corresponding mean obtained from the tubules perfused with Hg^{2+} plus GSH in the lumen and exposed to DMPS in the bath during the same 15 min of perfusion.

inorganic mercury is provided for the tubule. This protection appears to be linked to negligible rates of net absorption of inorganic mercury from the lumen and low levels of accumulation of mercuric ions. These findings most likely reflect a combination of extraction of cellular mercury during the bath-to-lumen *trans*-epithelial transport of DMPS and formation of nontransportable mercuric conjugates of DMPS after molecules of DMPS were transported rapidly from the bath into a lumen filled with mercuric ions.

The notion of formation of nontransportable mercuric conjugates of DMPS under conditions in which DMPS is being transported into a lumen containing mercuric ions is supported in part by the data obtained from the experiments in which 20 μM inorganic mercury and 80 μM DMPS were coperfused through the lumen. With 20 μM inorganic mercury and 80 μM DMPS together in aqueous solution, conditions strongly favored the formation of thermodynamically stable mercuric conjugates of DMPS (Ruprecht, 1997). When S2 segments were perfused through the lumen with presumed mercuric conjugates of DMPS, no tubular pathological characteristics were detected, and the rates of absorption and accumulation of inorganic mercury were also negligible or very low. These findings indicate not only that the S2 segments were most likely being perfused with mercuric conjugates of DMPS but also that these DMPS conjugates were not taken up at all from the lumen or they were taken up by a low-affinity and/or low-capacity system in these tubular segments.

It is also interesting that, when DMPS and inorganic mercury were applied simultaneously (presumably in the form of mercuric conjugates of DMPS) to the basolateral membranes of perfused S2 segments, relatively very little inorganic mercury was taken up and the tubular epithelial cells were afforded protection from the toxic effects of inorganic mercury. Our control data indicate that, when the basolateral surfaces of S2 segments are exposed to 20 μM inorganic mercury, there is very rapid uptake and accumulation of inorganic mercury and the perfused tubular segments become intoxicated and necrotic shortly after exposure. Therefore, based on the transport data from the bath-to-lumen flux experiments, it appears that mercuric conjugates of DMPS are also not taken up readily at the basolateral membrane. These findings are in contrast to a previously held notion that mercuric conjugates of DMPS might be transportable forms of inorganic mercury (particularly at the basolateral membrane). Although the present findings do not provide any information regarding the species of mercuric conjugates formed, it is clear that, whatever species were formed in the extracellular environment under the conditions used in the present study, the conjugates were not readily taken up at the luminal or basolateral membranes of S2 segments of proximal tubules.

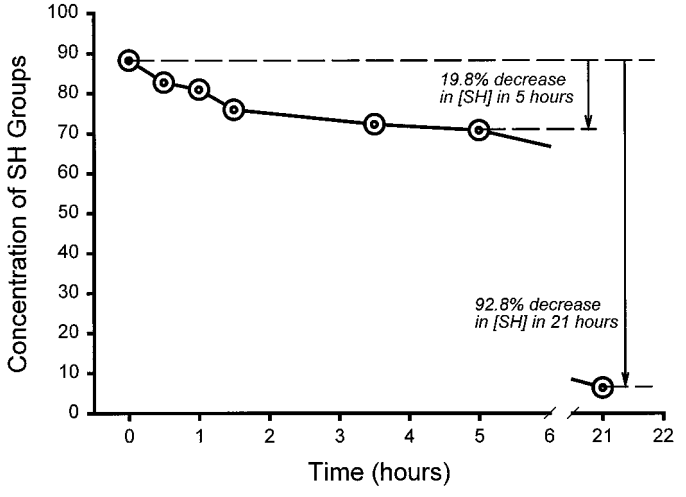

Fig. 8. Rate of oxidation of sulfhydryl (*SH*) groups on DMPS in perfusion medium at 25° (concentration: μM). The perfusion medium was identical to that used in the perfusion experiments, with the exception that no inorganic mercury was present. *Values*, mean ± standard error of 18 measurements. Standard errors were so small that they are not visible in this figure.

Previous *in vivo* and *in vitro* data indicate that DMPS, besides being filtered at the glomerulus, is secreted from the blood into the tubular lumen by the probenecid- and PAH-sensitive organic anion transport system (Stewart and Diamond, 1987, 1988; Klotzbach and Diamond, 1988). The sulfonate group on the terminal carbon of the propane skeleton affords DMPS a net negative charge at physiological pH, which is likely the primary contributing factor promoting the secretion of this molecule along the proximal tubule. Data from the present study provide additional evidence that DMPS is secreted in proximal tubular segments and the uptake of DMPS at the basolateral membrane is dependent on the activity of the organic anion transport system. When either 20 mM PAH or 4 mM glutarate was added to the bath, the inhibitory effects of DMPS on the uptake, accumulation, and toxicity of inorganic mercury were greatly diminished or eliminated. Inorganic mercury was taken up at levels near control levels, and cellular injury occurred along the perfused tubules. In other words, the levels of uptake, accumulation, and toxicity of inorganic mercury were comparable to levels detected in perfused S2 segments that were not exposed to DMPS at their basolateral membranes. It is likely that the inhibitory effects of glutarate on the therapeutic effects of DMPS were not only the result of competition for the dicarboxylic acid transporter but also the result of direct competition at the site of the organic anion transporter, which is consistent with the current known effects of glutarate (Pritchard and Miller, 1993; Dantzler, 1996). It is interesting that *in vivo* pretreatment of rats with glutarate also inhibits the basolateral uptake of administered inorganic mercury (Zalups and Barfuss, 1998), which indirectly implicates the actions of both the dicarboxylic acid transporter and the organic anion transporter in the basolateral uptake of some form of inorganic mercury in proximal tubules.

The transport data from the bath-to-lumen flux experiments are somewhat perplexing, because of the fact that some of the mercuric conjugates formed were likely in the form of one mercuric ion bonded to both sulfur atoms of one molecule of DMPS (Ruprecht, 1997). Because of the small size of this conjugate and the net negative charge it possesses, one could postulate that this complex might be transported by the organic anion transporter. However, our findings indicate that the bonding of inorganic mercuric ions to molecules of DMPS greatly influences the ability of proximal tubular cells to take up DMPS or inorganic mercury into the intracellular compartment.

One of the purported therapeutic actions of DMPS is to reduce the renal burden of mercury after exposure (Gabard, 1976; Planas-Bohne, 1981; Hursch *et al.*, 1985; Zalups, 1993;

Zalups and Lash, 1994; Ruprecht, 1997). Recent dispositional data from our laboratories strongly support this action of DMPS. Our data show clearly that, when DMPS is administered to rats 24 hr after a nontoxic dose of inorganic mercury, the total renal burden of inorganic mercury is reduced by >80% during the subsequent 24 hr (Zalups, 1993). Based on the findings in that study, it was postulated that the tremendous reduction in the renal burden of mercury was the result of extraction of inorganic mercury from the renal (proximal) tubular cells. Because the data from the present study indicate that mercuric conjugates of DMPS are not readily transported into proximal tubular cells, at either luminal or basolateral membranes, the only possible way in which DMPS can reduce the renal tubular burden of mercury after exposure to inorganic mercury is by some form of extraction of mercury during the *trans*-epithelial transport of DMPS. Indeed, our findings show that DMPS, when applied to the basolateral membrane, can rapidly and efficiently extract accumulated inorganic mercury and deliver it into the lumen of S2 segments of proximal tubules that are being perfused through the lumen with mercuric conjugates of GSH, which is a postulated form of inorganic mercury delivered to the lumen of proximal tubular segments *in vivo* (Zalups and Lash, 1994; Zalups, 1995). The findings that most convincingly support this hypothesis are the rates of luminal disappearance of inorganic mercury. In the same tubules perfused with mercuric conjugates of GSH, we show that the rates of disappearance of inorganic mercury change from positive values (averaging approximately 32 fmol/min/mm) to markedly negative values (averaging approximately −42 fmol/min/mm) almost immediately after DMPS is added to the bath. Based on additional data, such as a lack of cellular pathological changes and reasonably normal rates of intercellular leak of the volume marker throughout the experiment, the only reasonable explanation for the findings with these tubules is that DMPS extracted inorganic mercury from the proximal tubular cells and delivered it preferentially into the lumen in a form that is either not absorbed at all or not absorbed readily. These findings represent the first direct evidence for extraction of inorganic mercury from proximal tubular epithelial cells after the application of DMPS to the basolateral membrane. It should be stressed that we use the term "extraction" loosely in this article, because our data do not allow us to differentiate the fraction of mercury removed from within the intracellular compartment from the amount of mercury removed from the external surface of the plasma membrane. However, because of the tremendous reduction in the tubular burden of inorganic mercury detected both *in vivo* and *in vitro*, it is likely that the mercury extracted by DMPS represents pools of mercury from both intracellular and external membrane compartments.

In summary, our findings indicate that the therapeutic efficacy of DMPS in the nephropathy induced by inorganic mercury is in part linked to its transport at the basolateral membrane by the organic anion transport system and its ability to extract accumulated inorganic mercury from proximal tubular epithelial cells and then to preferentially deliver this mercury (in the form of a nonreabsorbable complex) into the tubular lumen, to promote the urinary excretion of mercury. Another therapeutic effect of DMPS is the prevention of significant proximal tubular uptake of inorganic mercury, from either the luminal fluid or blood, after the formation of mercuric conjugates of DMPS.

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