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Disposition of Inorganic Mercury Following Biliary Obstruction and Chemically Induced Glutathione Depletion: Dispositional Changes One Hour after the Intravenous Administration of Mercuric Chloride

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Influences of biliary obstruction and systemic depletion of glutathione (GSH) on the disposition of a low nontoxic iv dose of inorganic mercury were evaluated in rats in the present study. Specifically, the disposition of mercury in the kidneys, liver, small and large intestines, and blood was assessed 1 h after the injection of 0.5 μmol/kg mercuric chloride in control rats and rats pretreated with acivicin, buthionine sulfoximine (BSO), or diethylmaleate (DEM) that did or did not undergo acute biliary ligation prior to the injection of mercury. Among the groups that did not undergo biliary ligation, the pretreatments used to alter GSH status systemically had varying effects on the disposition of inorganic mercury in the kidneys, liver, intestines, and blood. Biliary ligation caused the net renal accumulation of mercury to decrease under all pretreatment conditions. By contrast, biliary ligation caused significant increases in the hepatic burden of mercury in all pretreatment groups except the acivicin-pretreated group. Blood levels of mercury also increased as a result of biliary ligation, regardless of the type of pretreatment used. Evidence for a secretory-like movement of mercury into the lumen of the intestines is also provided in the animals that underwent biliary ligation. The present findings indicate that biliary ligation combined with methods used to alter GSH status systemically have additive effects with respect to causing reductions in the net renal accumulation of mercury. In addition, the findings indicate that at least some fraction of the renal accumulation of inorganic mercury is linked mechanistically to the hepatobiliary system.

We recently investigated the effects of three different ways of altering systemic glutathione (GSH) status on the disposition of inorganic mercury in rats 1 h after the administration of a nonnephrotoxic dose of mercuric chloride (Zalups and Lash, 1997a). Pretreatment of rats with buthionine sulfoximine (BSO) to inhibit the γ-glutamylcysteine synthetase, diethylmaleate (DEM) to deplete intracellular GSH by conjugation, or acivicin to inhibit γ-glutamyltransferase caused a very rapid and significant decrease in the concentration and content of GSH in both the liver and kidneys. Moreover, pretreatment with DEM and acivicin, but not BSO, caused the renal accumulation of mercury to be diminished significantly, relative to that in untreated control animals. On the basis of these findings, we concluded that the mechanism by which systemic GSH is altered plays an important role in the acute rapid phase of the renal uptake and accumulation of administered inorganic mercury.

In another recent study, it was shown that acute obstruction or diversion of bile flow resulted in significantly diminished renal uptake and accumulation of administered inorganic mercury in rats (Zalups and Barfuss, 1996). It was postulated that the decreased renal accumulation of mercury was related to putative alterations in thiol status, in particular GSH status, induced by preventing bile from entering into the small intestine. Efflux of GSH from the liver into the bile is the primary mechanism of hepatic turnover of GSH and is the principal source of GSH that is translocated through both enterohepatic and renal-hepatic circulation to other tissues. (Lash *et al.*, 1988). As such, altering the biliary delivery of hepatically synthesized GSH to the small intestine should have a significant effect on GSH status in extrahepatic tissues. It was also demonstrated that some form of intestinal secretion of mercury occurred in the animals whose bile duct had been ligated or cannulated.

Since GSH status had been implicated both directly and indirectly in the renal dispositional findings of the two studies mentioned above, we used the present study to test the hypothesis that the effects of obstructing bile flow and systemic alteration of GSH (by the three means described above) are additive with respect to the renal uptake and/or net accumulation of administered inorganic mercury. As part of the experiments designed to test this hypothesis, we also evaluated the combined effects of biliary ligation and GSH depletion on the disposition of inorganic mercury in the liver, blood, and intestines.

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MATERIALS AND METHODS

Animals and groups. Thirty-six male Sprague–Dawley rats were used to study the disposition of inorganic mercury and 24 rats were used to assess the effect of chemically induced depletion of GSH and biliary ligation on the disposition of GSH in the kidneys and liver. All rats were purchased from Harlan Sprague–Dawley (Indianapolis, IN) at a weight of 175 to 200 g. After 3 to 4 days of acclimation, the animals were separated into groups of four or five.

There were two main surgical groups, one that underwent acute biliary ligation and the other that served as control. We have determined in a preliminary study that there was no difference in the disposition of mercury between sham-operated and nonoperated control animals, and therefore, chose to use non-operated control animals in the present study. In the experiments in which the disposition of inorganic mercury was assessed, each of the two surgical groups was subdivided into four types of treatment groups. One type was pretreated with normal saline and served as a control, one type was pretreated with acivicin (to inhibit the enzyme γ-glutamyltransferase), one type was pretreated with BSO to deplete renal and hepatic GSH, and the last type was pretreated with DEM as a second method to deplete renal and hepatic GSH. In the experiments in which GSH status in the kidneys and liver was assessed, each of the two surgical groups was subdivided into three types of treatments. One type served as a control, one type was pretreated with BSO, and the third type was pretreated with DEM.

During all stages of the present study, the animals were allowed water and a commercial laboratory diet for rats *ad libitum.*

Surgical procedures. Animals designated to undergo biliary ligation were first anesthetized with an injection of ketamine (70 mg/kg im) and xylazine (6 mg/kg im), and then a midline incision was made through the skin and abdominal muscles. Once the abdominal cavity had been opened, the intestines were moved and exteriorized to the left side of the animal until the bile duct could be identified. The bile duct was isolated from its origin in the liver to its insertion into the small intestine by blunt dissection. Then two 4-0 silk ligatures were tied securely around the bile duct near its origin in the liver. After the intestines were placed back into their proper position, the abdominal muscles were sewn together with sterile 4-0 silk suture and the skin was approximated with sterile 9-mm wound clips.

Pretreatments. As mentioned above, acivicin was administered to inhibit the activity of the enzyme γ-glutamyltransferase in the kidneys and liver. The injection protocol used in the animals pretreated with acivicin is a slight modification of the one established by Scott and Curthoys (1987), which results in the inhibition of approximately 97% of the activity of γ-glutamyltransferase in the kidneys. First, the animals received a 10-mg/kg ip dose of acivicin in 2.0 ml/kg normal saline (0.9% w/v aqueous sodium chloride). Ninety minutes later, the animals received a second 10-mg/kg dose of acivicin that was administered into the left femoral vein (while they were anesthetized lightly with ether). Sixty minutes after the second dose, the animals received a nontoxic 0.5 μmol/kg iv dose of mercuric chloride.

BSO and DEM were chosen to deplete GSH in the kidneys and liver because depletion could be attained by different mechanisms. Depletion of GSH following pretreatment with BSO is accomplished by inhibition of γ-glutamylcysteine synthetase (Griffith and Meister, 1979), which is the rate-limiting enzyme involved in the intracellular synthesis of GSH. Pretreatment with DEM results in depletion of GSH by formation of DEM–GSH conjugates. The injection protocols used to pretreat rats with BSO or DEM were slight modifications of ones we used previously (Zalups and Barfuss, 1996). In brief, rats pretreated with BSO were given a 2.0-mmol/kg dose iv in 4.0 ml/kg normal saline. Rats that were pretreated with DEM received a 3.37-mmol/kg dose ip in 2.0 ml/kg corn oil. Baggett and Berndt (1986) have shown that this type of pretreatment with DEM causes the nonprotein thiol content in the kidneys and liver to decrease by approximately 40 and 68%, respectively. Two hours after pretreatment with either BSO or DEM, the animals received the nonnephrotoxic 0.5-μmol/kg iv dose of mercuric chloride. On examination after the administration of acivicin, BSO, or DEM, the gross structural characteristics of the kidneys appeared normal 1 h after the administration of the 0.5-μmol/kg dose of mercuric chloride. This suggests that no toxic effects occurred as a result of the pretreatments used during the period studied.

Injection of inorganic mercury. All groups of control, acivicin-pretreated, BSO-pretreated, and DEM-pretreated rats were administered a 0.5-μmol/kg dose of mercuric chloride into the femoral vein while under light anesthesia induced by ether. By the time each animal was injected with inorganic mercury, it had fully recovered from surgical anesthesia induced by ketamine and xylazine. In addition, inorganic mercury was administered to each animal while it was anesthetized lightly with ether (which lasted no more than 30 s). Radioactive inorganic mercury in the form of mercuric chloride ($^{203}HgCl_2$, specific activity = 30 mCi/mg; Buffalo Materials Corp., Buffalo, NY) was added to the injection solution containing nonradioactive mercury. The injection solution was designed to deliver 0.5 μmol Hg^{2+}/kg and 4 μCi $^{203}Hg^{2+}$/kg in 2.0 ml normal saline (0.2 ml injection volume/100 g body wt).

Acquisition of tissues and determination of the content of mercury in the tissues. One hour after the injection of the mercuric chloride, animals were anesthetized with a 100-mg/kg ip dose of sodium pentobarbital. Once the animals were anesthetized, two 1-ml samples of whole blood were obtained from the inferior vena cava. One milliliter of whole blood was placed and sealed in a pre-weighed 12 × 75 mm, round-bottom, gamma-counting tube. The other 1.0 ml of whole blood was centrifuged at 10,000*g* to separate the cellular fraction of blood from the plasma. Both plasma and cellular fractions were placed individually and sealed in gamma-counting tubes. After the blood had been obtained, the kidneys, liver, small intestine, and large intestine were removed, cleared of fat and connective tissue, and weighed quickly. Each of the two kidneys was cut along the transverse plane. One half of each kidney was placed and sealed in a preweighed gamma-counting tube. A 3-mm section of kidney was sliced away from the midregion of the remaining half of the left kidney and samples of cortex, outer and inner stripes of the outer medulla, and inner medulla were obtained. A 1-g sample of liver was also obtained. The luminal contents of both the small and large intestine were washed out by perfusing approximately 6-ml of 0.9% (w/v) aqueous sodium chloride through the lumen via a 10-cc syringe attached to one of the ends of the isolated intestine. The saline solution coming out of the opposite end of the intestine was collected in 16 × 90 mm polypropylene tubes, which were also used as gamma-counting tubes. After the luminal contents were washed out of the isolated segments of the intestines, the intestinal segments were also placed and sealed individually in the polypropylene gamma-counting tubes.

The amount of radioactivity in the samples of tissues and injection solution (standards) was determined by counting the samples in a 1282 Compugamma CS deep-well gamma spectrometer that is equipped with a 3-inch sodium iodide crystal (Wallac, Gaithersburg, MD) and operates at a counting efficiency of approximately 50% for $^{203}Hg^{2+}$. The content of mercury in the samples was calculated by dividing the activity of $^{203}Hg^{2+}$ (dpm) in the sample by the specific activity of $^{203}Hg^{2+}$ (dpm/nmol) in the injection solution. Concentrations of mercury in the tissues are expressed as percent of the administered dose per gram of tissue and the content of mercury in organs is expressed simply as a percent of the administered dose. The total volume of blood in rats was estimated to be ~6% of body weight.

The 1-h time point after the administration of mercuric chloride was chosen for measurement of the disposition of inorganic mercury because the kinetics for the uptake of mercury in the kidney and liver are most rapid during this period. Approximately 80 to 85% of the total renal burden of mercury that is normally recovered in 24 h is taken up by the kidney during this initial hour. Moreover, the 1-h time point was chosen since we wanted to evaluate the effects of depletion of GSH on the disposition of mercury at a time when it was most likely that GSH status would not have recovered from the changes induced by the administration of acivicin, BSO, or DEM.

Assessment of GSH status in the kidneys and liver. In the animals used to assess GSH status, the kidneys and liver were removed at the termination of the corresponding pretreatment periods. After the kidneys and liver had been removed and weighed quickly, samples of kidney and liver were homogenized in 10 ml of 1 mM bathophenanthroline disulfonate (used as an antioxidant) and

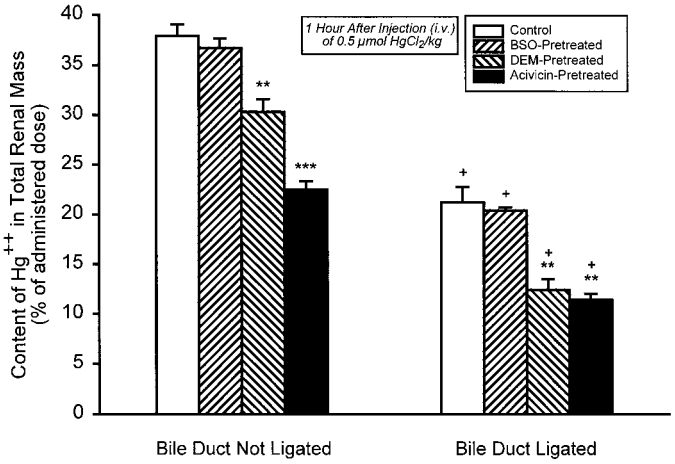

FIG. 1. Effect of acute biliary obstruction (ligation) on the content of mercury (% of administered dose) in the total renal mass of control rats and rats pretreated with acivicin, BSO, or DEM 1 h after the iv injection of a 0.5-μmol/kg dose of mercuric chloride. The pretreatments used represent three different means of depleting GSH systemically. Refer to Materials and Methods for details on pretreatments and handling of tissues. Each value represents the mean ± SE for four animals in the groups that underwent biliary ligation and five animals in the remaining groups. **Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. ***Significantly different ($p < 0.05$) from each of the remaining means for the corresponding groups treated surgically in the same manner. +Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation but was pretreated in the same manner.

10% (v/v) perchloric acid. Acid extracts of the homogenates were derivatized with iodoacetic acid and 1-fluoro-2,4-dinitrobenzene for analysis of GSH by high-performance liquid chromatography (HPLC). The *S*-carboxymethyl-*N*-dinitrophenyl derivative of GSH was separated on a 10-μm μBondapak amine column with gradient elution (methanol-acetate solvent system) and was detected by absorbance at 365 nm. Quantitation of GSH in samples was performed by integration. The actual amount of GSH in the combined total renal mass and liver is expressed in μmol/organ(s).

Statistical analyses. Statistical differences between means, of any parameter measured, for the groups of rats studied were assessed using a two-way analysis of variance followed by Tukey's multiple comparison procedure. Data expressed as a percentage of total were first normalized using the arcsine transformation before performing any parametric statistical procedure. The arcsine transformation takes the arcsine of the square root of the decimal fraction of the percent score. The level of significance for any of the statistical procedures used was chosen *a priori* to be $p < 0.05$.

RESULTS

Content of Mercury in the Total Renal Mass

Bile duct not ligated. One hour after the injection of the 0.5-μmol/kg iv dose of mercuric chloride, the amount of mercury in the total renal mass was not statistically different between the control rats and BSO-pretreated rats that did not undergo biliary ligation (Fig. 1). By contrast, the content of mercury in the total renal mass was significantly lower in the corresponding DEM-pretreated rats that did not undergo biliary ligation. In the acivicin-pretreated rats that did not undergo biliary ligation, the content of mercury in the total renal mass was significantly lower than that in the rats of any of three corresponding groups treated surgically in the same manner. In fact the renal burden of mercury in the acivicin-pretreated rats was approximately 38% lower than that in the control rats.

Bile duct ligated. In the control and BSO-pretreated rats that underwent biliary ligation, the content of mercury in the total renal mass was approximately 43% lower than that in the corresponding control rats and BSO-pretreated rats that did not undergo biliary ligation (Fig. 1). Interestingly, the content of mercury in the total renal mass of the DEM-pretreated rats and acivicin-pretreated rats that underwent biliary ligation was approximately 57 and 49% lower, respectively, than that in the corresponding rats pretreated in the same manner that did not undergo biliary ligation. There was no significant difference in the total renal burden of mercury between the control rats and the BSO-pretreated rats or between the acivicin-pretreated rats and the DEM-pretreated rats that underwent biliary ligation. However, among the groups that underwent biliary ligation, the content of mercury in the total renal mass of either the rats pretreated with DEM or acivicin was significantly lower than that in the control rats or the rats pretreated with BSO.

Intrarenal Distribution of Mercury

Bile duct not ligated. Among the rats that did not undergo biliary ligation, the concentration of mercury in the renal cortex in the BSO-pretreated, DEM-pretreated, and acivicin-pretreated rats was significantly lower than that in the control rats (Fig. 2A). Moreover, the renal cortical concentration of mercury in the acivicin-pretreated rats was significantly lower than that in any of the other three corresponding groups.

In the DEM-pretreated rats that did not undergo biliary ligation, the concentration of mercury in the renal outer stripe of the outer medulla was significantly lower than that in any of the other three corresponding groups of rats (Fig. 2B). No other significant differences in the concentration of mercury in the outer stripe of the outer medulla were detected among the four groups of rats that did not undergo biliary ligation.

Bile duct ligated. When comparing the renal concentration of mercury between the corresponding two groups of rats pretreated in the same manner, the renal concentration of mercury in the group that underwent biliary ligation was significantly lower than that in the corresponding group that did not undergo acute biliary ligation (Fig. 2A). Among the groups that underwent biliary ligation, the renal concentration of mercury was significantly lower in the DEM-pretreated and acivicin-pretreated rats than in the control rats or BSO-pretreated rats. Moreover, similar to the groups of rats that did not undergo biliary ligation, the renal concentration of mercury

among the rats that underwent biliary ligation was lowest in the acivicin-pretreated group.

Between the paired groups of rats that were pretreated in the same manner, the concentration of mercury in the renal outer stripe of the outer medulla in the group that underwent biliary ligation was significantly lower than that in the corresponding group that did not undergo acute biliary ligation (Fig. 2B). As was the case among the groups that did not undergo biliary ligation, the concentration of mercury in the renal outer stripe of the outer medulla of the DEM-pretreated rats that underwent biliary ligation was significantly lower than that in any of the other three corresponding groups of rats. In addition, no other significant differences in the concentration of mercury in the outer stripe of the outer medulla were detected among the four groups of rats that underwent biliary ligation.

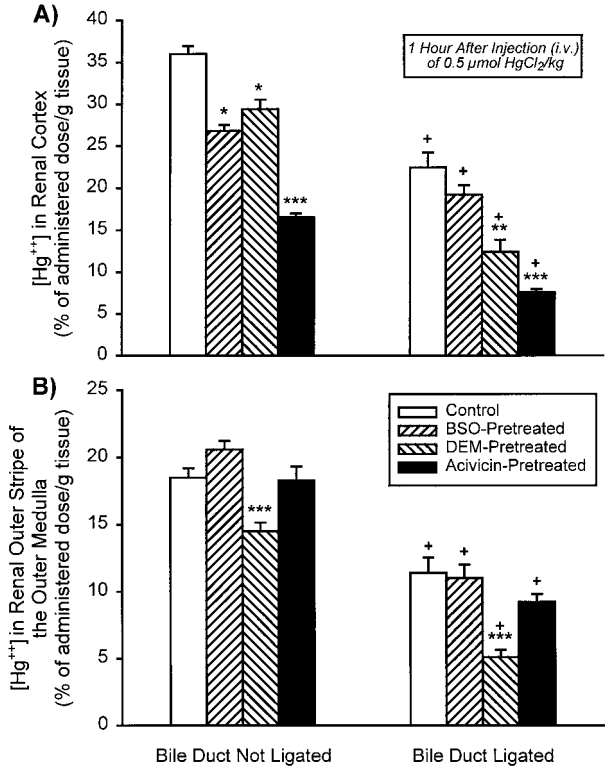

FIG. 2. Effect of acute biliary obstruction (ligation) on the concentration of mercury (% of administered dose/g tissue) in the renal cortex (A) and outer stripe of the outer medulla (B) of control rats and rats pretreated with acivicin, BSO, or DEM 1 h after the iv injection of a 0.5-μmol/kg dose of mercuric chloride. The pretreatments used represent three different means of depleting GSH systemically. Refer to Materials and Methods for details on pretreatments and handling of tissues. Each value represents the mean ± SE for four animals in the groups that underwent biliary ligation and five animals in the remaining groups. **Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. ***Significantly different ($p < 0.05$) from each of the remaining means for the corresponding groups treated surgically in the same manner. +Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation but was pretreated in the same manner.

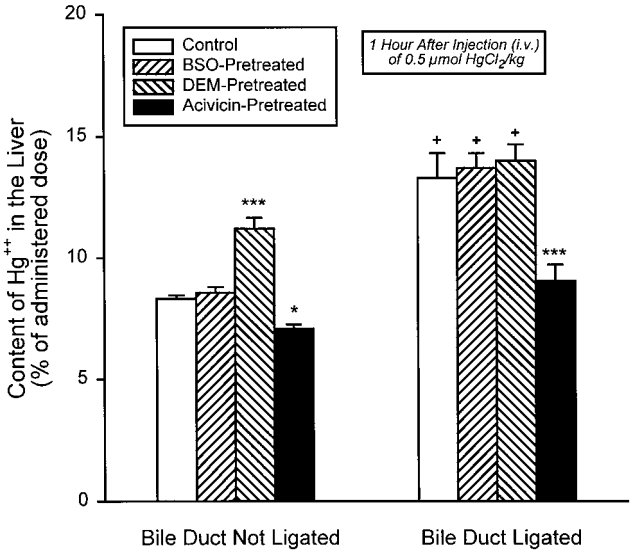

FIG. 3. Effect of acute biliary obstruction (ligation) on the content of mercury (% of administered dose) in the liver of control rats and rats pretreated with acivicin, BSO, or DEM 1 h after the iv injection of a 0.5-μmol/kg dose of mercuric chloride. The pretreatments used represent three different means of depleting GSH systemically. Refer to Materials and Methods for details on pretreatments and handling of tissues. Each value represents the mean ± SE for four animals in the groups that underwent biliary ligation and five animals in the remaining groups. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats that was treated surgically in the same manner. ***Significantly different ($p < 0.05$) from each of the remaining means for the corresponding groups treated surgically in the same manner. +Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation but was pretreated in the same manner.

Content of Mercury in the Liver

Bile duct not ligated. Significant changes in the disposition of mercury in the liver were detected among the four groups of rats that did not undergo biliary ligation 1 h after the iv injection of the 0.5-μmol/kg dose of mercuric chloride (Fig. 3). The hepatic content of mercury in the DEM-pretreated rats was significantly greater than that in any of the other three corresponding groups. By contrast, the hepatic content of mercury in the acivicin-pretreated rats was significantly less than that in any of the other three corresponding groups.

Bile duct ligated. In the paired groups of control, BSO-pretreated and DEM-pretreated rats, the content of mercury in the liver was significantly greater in the corresponding rats that underwent biliary ligation (Fig. 3). Among the four groups of rats that underwent biliary ligation, the hepatic content of mercury was significantly lower in the acivicin-pretreated group than in the other three corresponding groups.

Disposition of Mercury in the Blood

No significant differences in the content of mercury in blood (Fig. 4A) and in the distribution of mercury between the

plasma and cellular fractions of blood (Fig. 4B) were detected among the four groups of rats that did not undergo biliary ligation.

Mercury content in the blood of each of the four groups of rats that underwent biliary ligation was significantly greater than that of their corresponding paired group of rats that were pretreated in the same way but that did not undergo biliary ligation (Fig. 4A). In addition, among the groups that underwent biliary ligation, the content of mercury in the blood was significantly greater in the DEM-pretreated rats than in any of the other three corresponding groups.

Between the two groups of rats pretreated with BSO, the percent of mercury present in the plasma was significantly greater in the group that underwent biliary ligation than in the group that did not. The only other statistical difference detected among the groups that underwent biliary ligation was between the BSO-pretreated and DEM-pretreated groups. More mercury was present in the plasma of the BSO-pretreated rats than in the DEM-pretreated rats.

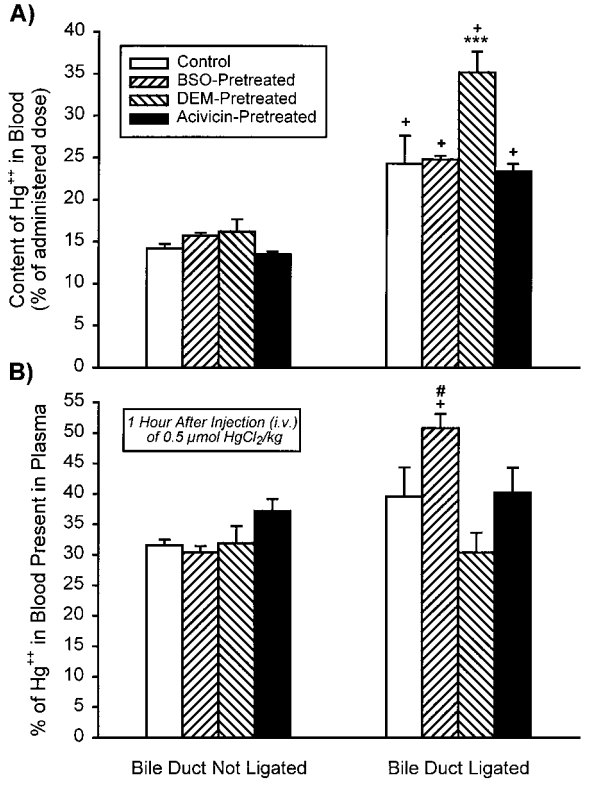

FIG. 4. Effect of acute biliary obstruction (ligation) on the content of mercury (% of administered dose) in the blood (A) and percent of mercury in blood present in plasma (B) of control rats and rats pretreated with acivicin, BSO, or DEM 1 h after the iv injection of a 0.5-μmol/kg dose of mercuric chloride. The pretreatments used represent three different means of depleting GSH systemically. Refer to Materials and Methods for details on pretreatments and handling of tissues. Each value represents the mean ± SE for four animals in the groups that underwent biliary ligation and five animals in the remaining groups. ***Significantly different ($p < 0.05$) from each of the remaining means for the corresponding groups treated surgically in the same manner. +Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation but was pretreated in the same manner. #Significantly different ($p < 0.05$) from the mean for the corresponding group of rats that underwent biliary ligation and was pretreated with DEM.

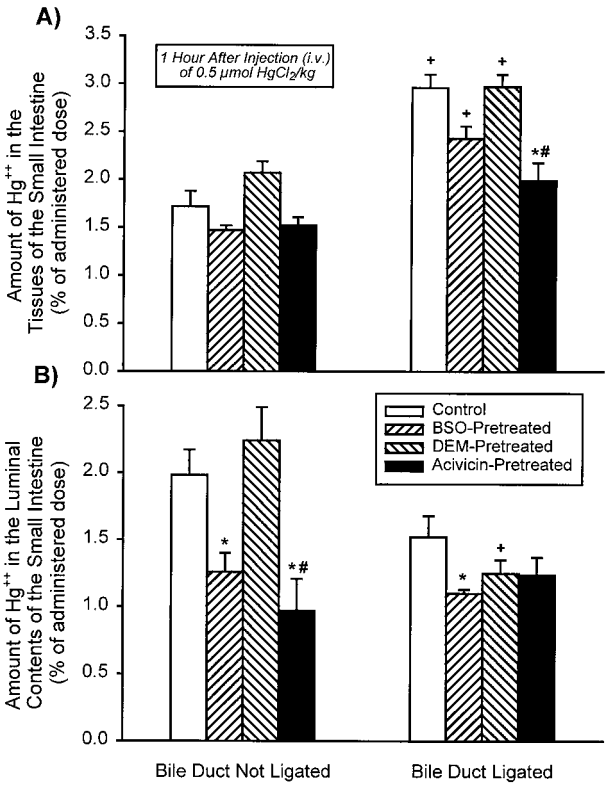

FIG. 5. Effect of acute biliary obstruction (ligation) on the content of mercury (% of administered dose) in the tissues (A) and luminal contents (B) of the small intestine of control rats and rats pretreated with acivicin, BSO, or DEM 1 h after the iv injection of a 0.5-μmol/kg dose of mercuric chloride. The pretreatments used represent three different means of depleting GSH systemically. Refer to Materials and Methods for details on pretreatments and handling of tissues. Each value represents the mean ± SE for four animals in the groups that underwent biliary ligation and five animals in the remaining groups. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats that was treated surgically in the same manner. #Significantly different ($p < 0.05$) from the means for the corresponding group of control rats and the group of rats pretreated with DEM that were treated surgically in the same manner. +Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation but was pretreated in the same manner.

Disposition of Mercury in the Small Intestine

Significant differences in the content of mercury in the tissue compartment of the small intestine were not detected among the four groups of rats that were not treated surgically (Fig. 5A). By contrast, significant differences were detected between corresponding paired groups pretreated in the same manner. With the exception of groups pretreated with acivicin, the content of mercury in the tissue compartment of the small intestine was significantly greater in the rats that underwent biliary ligation than in the corresponding group pretreated in

the same manner that did not undergo biliary ligation. Among the four groups that underwent biliary ligation, the content of mercury in the tissues of the small intestine was significantly lower in the group pretreated with acivicin than in the control group or the group pretreated with DEM.

Among the four groups of rats that were not treated surgically, a significantly lower amount of mercury in the luminal contents of the small intestine was detected in the groups pretreated with BSO or acivicin than in the other two groups (Fig. 5B). Significant effects of biliary ligation on the content of mercury in the lumen of the small intestine were detected only in the groups pretreated with DEM. The content of mercury in the lumen of the small intestine of the DEM-pretreated rats that underwent biliary ligation was significantly lower than that in the DEM-pretreated rats that were not treated surgically. Morever, among the four groups that did undergo biliary ligation, the content of mercury in the lumen was significantly lower in the BSO-pretreated group than in the corresponding control group.

Disposition of Mercury in the Large Intestine

There were no significant differences in the content of mercury in the tissues of the large intestine among the eight groups of rats in the present study (Fig. 6A).

In the BSO-pretreated, DEM-pretreated, and acivicin-pretreated rats that were not treated surgically, the content of mercury in the luminal contents of the large intestine was significantly less than that in the corresponding control rats. Moreover, the amount of mercury in the lumen of the large intestine of the DEM-pretreated rats was significantly less than that of any of the other three corresponding groups of rats (Fig. 6B).

Biliary ligation had an effect on the disposition of mercury in the lumen of the large intestine in the control rats and the BSO-pretreated rats. Between the two groups of control rats and the two groups of DEM-pretreated rats, the content of the mercury in the luminal contents of the large intestine was significantly less than that in the corresponding rats that underwent biliary ligation.

Among rats that underwent biliary ligation, the luminal content of mercury in the large intestine in the BSO-pretreated, DEM-pretreated, and acivicin-pretreated groups was significantly less than that in the corresponding control rats. No other significant differences in the luminal content of mercury in the large intestine were detected among these four groups.

Recovery and Distribution of Mercury in Tissues

As shown in the figures, the overall recovery of the administered dose of mercury in the combined kidneys, liver, blood, the tissue of the small and large intestine, and the luminal contents of the small and large intestine was similar for control, BSO-pretreated, and DEM-pretreated animals from both bile duct-ligated and nonligated groups. Values for recovery ranged

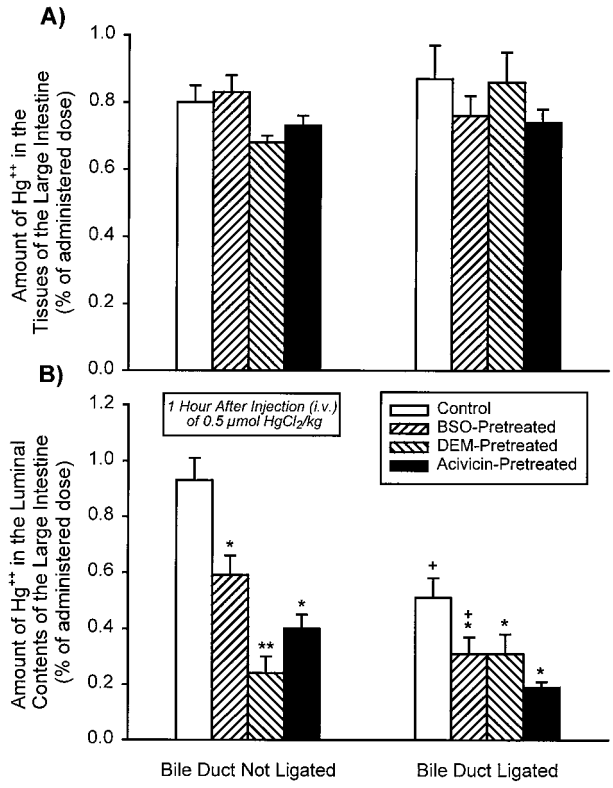

FIG. 6. Effect of acute biliary obstruction (ligation) on the content of mercury (% of administered dose) in the tissues (A) and luminal contents (B) of the large intestine of control rats and rats pretreated with acivicin, BSO, or DEM 1 h after the iv injection of a 0.5-μmol/kg dose of mercuric chloride. The pretreatments used represent three different means of depleting GSH systemically. Refer to Materials and Methods for details on pretreatments and handling of tissues. Each value represents the mean $\pm$ SE for four animals in the groups that underwent biliary ligation and five animals in the remaining groups. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats that was treated surgically in the same manner. **Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO that were treated surgically in the same manner. +Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo biliary ligation but was pretreated in the same manner.

between approximately 63 and 68%. The remaining amount of mercury not accounted for by these measurements was likely present in other tissues and/or was excreted in the urine. In contrast to these values, only 46 to 49% of the administered mercury was recovered in the two groups of rats that were pretreated with acivicin. Much of the difference in the recovery of mercury in the acivicin-pretreated rats is likely due to enhanced urinary excretion of mercury, which has been shown to occur in a previous study (Zalups, 1995).

The other major difference in the distribution of mercury was that, as a result of biliary obstruction, a much larger proportion of the dose of mercury was recovered in the blood and a much smaller fraction of mercury was recovered in the kidneys. Ligation of the bile duct also produced an increase in the recovery of mercury in the tissues of the small intestine,

TABLE 1

Effect of Biliary Ligation and Treatment with Depletors of GSH on the Content of GSH in the Total Renal Mass and Liver of Rats

Treatment	Control	Bile duct ligated
Content of GSH (μmol) in the Kidneys		
Control	9.39 ± 0.38	15.1 ± 0.8[c]
BSO	3.27 ± 0.21[a]	3.53 ± 0.39[a]
DEM	6.58 ± 0.34[a,b]	5.00 ± 0.48[a,c]
Content of GSH (μmol) in the Liver		
Control	81.5 ± 2.1	74.3 ± 2.1[c]
BSO	47.5 ± 2.9[a]	54.9 ± 3.1[a,c]
DEM	30.6 ± 1.6[a,b]	18.0 ± 1.4[a,b,c]

Note. Values represent means ± SE for four rats. Treatments were not carried out until all animals had recovered fully from anesthesia used for surgery. Control rats received 2.0 ml/kg iv normal saline. The BSO-treated rats received a 2.0-mmol/kg dose of BSO iv in 4.0 ml/kg normal saline. Rats treated with DEM received a 3.37-mmol/kg dose of DEM ip in 2.0 ml/kg corn oil. Kidneys and liver were removed from all animals 2 h after treatment.

[a] Significantly different ($p < 0.05$) from the mean for the control group within the same set of surgically treated animals.

[b] Significantly different ($p < 0.05$) from the mean for the BSO-treated group within same set surgically treated of animals.

[c] Significantly different ($p < 0.05$) from corresponding treatment group of rats whose bile duct had not been ligated.

suggesting that differences in enterohepatic circulation may cause alterations in the intestinal handling of inorganic mercury.

Effect of Biliary Ligation and Treatment with BSO or DEM on GSH Status in the Kidneys and Liver

Among the three groups of rats that did or did not undergo biliary ligation, the content of GSH in the total renal mass was significantly lower in the rats treated with BSO or DEM than in the corresponding control rats (Table 1). The content of GSH in the total renal mass was significantly greater in the control group that underwent biliary ligation than in the corresponding control group that did not undergo biliary ligation. Between the two groups of rats treated with DEM, the content of GSH in the kidneys was significantly lower in the group that underwent biliary ligation. By contrast, the content of GSH in the kidneys of the rats that had undergone biliary ligation and were treated with BSO did not differ significantly from that in the corresponding group of control rats treated with BSO.

As was the case for the renal content of GSH, the content of GSH in the liver was also significantly lower in the rats treated with BSO or DEM than in the corresponding control rats, regardless of whether the three groups had undergone biliary ligation (Table 1). Unlike with the renal content of GSH, the content of GSH in the liver of the control rats that had undergone biliary ligation was statistically lower than that of the control rats that had not undergone biliary ligation. Of the two groups of rats treated with DEM, the content of GSH in the liver was significantly lower in the group that underwent biliary ligation. No significant differences in the hepatic content of GSH were detected between the two groups of rats treated with BSO.

DISCUSSION

Pretreatment of normal rats with acivicin or DEM, but not BSO, prior to the administration of a 0.5-μmol/kg dose of mercuric chloride, was shown to cause a significant decrease in the net accumulation of inorganic mercury during the initial hour after treatment. The decreased net renal accumulation of mercury seems to be due primarily to changes in the disposition of mercury in the renal cortex. Only in animals pretreated with DEM was a decrease in the concentration of mercury detected in an additional renal zone, namely the outer stripe of the outer medulla. In general, the renal dispositional findings in the nonsurgically treated animals of the present study are consistent with the findings from our previous study in which normal rats were pretreated similarly (Zalups and Lash, 1997a).

There were, however, a few minor differences between some of the dispositional findings of the present study and those from our previous study. For example, unlike in our previous study, DEM pretreatment had less of an effect on reducing the net accumulation of inorganic mercury than did pretreatment with acivicin. It is likely that the differences in dispositional findings between the two studies are due to a modification in two of the methods used to deplete GSH. In the present study, animals pretreated with either BSO or DEM did not receive an initial priming dose of DEM 24 h prior to pretreatment, which was used in our previous study and likely had an added effect on the magnitude of reduction in the renal burden of inorganic mercury. In support of this contention, are the findings of Baggett and Berndt (1986), who demonstrated that nonprotein thiol content in the kidneys decreased by approximately 60% in rats pretreated with two doses of DEM (as in our previous study) while it decreased by only about 40% in rats pretreated with a single dose (as in the present study). Further support for this contention comes from the present study. We showed in surgical control animals that pretreatment with BSO caused significantly greater reductions in the content of GSH in the total renal mass than did treatment with DEM. Thus, it appears that the less pronounced effects of DEM pretreatment on the renal disposition of mercury detected in the present study relates to the lesser effect of the single pretreatment of DEM on GSH status in the kidneys.

Biliary ligation also caused a net reduction in the renal burden of inorganic mercury. This is consistent with the findings from a recent study of ours in which obstruction or diversion of biliary outflow also caused the renal uptake and/or accumulation of inorganic mercury to be decreased in rats

(Zalups and Barfuss, 1996). The findings from that study led us to postulate that obstruction or diversion of biliary flow causes a reduction in the renal burden of inorganic mercury by a mechanism that may involve alterations of thiol status (mainly GSH status) in systemic circulation. A major mechanism by which hepatically synthesized GSH is recycled involves its secretion into the bile with subsequent intestinal degradation and absorption of its constituent amino acids (Abbott and Meister, 1986; Cornell and Meister, 1976; Lash *et al.,* 1988). Therefore, it was speculated that preventing hepatically synthesized GSH from entering into the small intestine would lead to significant alterations in plasma thiol status, as shown in a study in which intestinal GSH was depleted in mice (Martensson *et al.,* 1990). This in turn could affect significantly renal and hepatic disposition of administered inorganic mercury.

It is somewhat perplexing that biliary ligation caused the content of GSH in the kidneys to increase significantly, while it had no apparent effect on the hepatic content of GSH. With increased renal content of GSH, one might speculate that the renal accumulation of mercury might increase. However, this was clearly not the case. Unfortunately, our present data do not allow us to comment on the effect of biliary ligation on the status of GSH and other thiols in the plasma. Based on the present findings, however, it appears that the mechanisms involved in the altered renal accumulation of mercury induced by biliary ligation are not as straightforward as initially thought.

In addition to causing reductions in the net renal accumulation of mercury, biliary ligation appears to have an additive effect on the renal disposition of mercury when it was combined with chemical pretreatments used to deplete GSH systemically. Our findings indicate that, regardless of the mechanism by which the depletion of GSH (in the kidneys and liver) is induced chemically, biliary ligation leads to further reductions in the renal burden of mercury in an additive manner. The additive effects detected lead one to suggest that mechanisms involved in the reduced renal accumulation of mercury induced by biliary ligation are different from those involved in the reduced renal accumulation of mercury that occurs following chemical treatments used to deplete renal and hepatic GSH.

The net effects of biliary ligation on the renal disposition of mercury appear to be due to changes in the disposition of mercury in both the renal cortex and the outer stripe of the outer medulla. One explanation for the additive effects of biliary ligation and pretreatment with DEM or acivicin on the renal disposition of mercury is that each pretreatment inhibits a separate mechanism involved in the renal uptake and/or accumulation of inorganic mercury. On the other hand, it may be that there is only one mechanism responsible for the changes detected, but that this mechanism is influenced in an additive manner by biliary ligation and pretreatment with either acivicin or DEM. Whichever may be the case, it should be pointed out that the combined effects of biliary ligation and treatment with either BSO or DEM on renal GSH status were not, however, consistent with the apparent additive effects seen in the disposition of mercury. Therefore, at least during the initial hour after the exposure to mercury, additional factors besides GSH status play(s) a role in the disposition of mercury in the kidneys.

Our renal dispositional data from the rats that underwent biliary ligation also support the hypothesis that the renal uptake and/or accumulation of inorganic mercury is linked in part to some aspect of hepatobiliary metabolism (presumably of thiols such as GSH and cysteine). Other investigators have postulated that hepatically synthesized GSH is linked in part to some aspects of the renal accumulation of inorganic mercury (Tanaka *et al.,* 1990). These investigators have demonstrated in mice that the renal accumulation of inorganic mercury is diminished significantly when 1,2-dichloro-4-nitrobenzene, which purportedly depletes GSH specifically in the liver, is administered prior to the administration of mercuric chloride. If a fraction of hepatically synthesized GSH is involved in some aspect of the renal uptake of mercury, then it would seem logical to postulate that perhaps some of the diminution in the renal burden of mercury detected following GSH depletion (induced by DEM and acivicin) is linked mechanistically to the depletion of GSH in the liver.

To place some of the present renal dispositional findings into perspective, there is a large body of evidence implicating the involvement of some form of mercuric conjugates of GSH (and cysteine) in the luminal and basolateral uptake of inorganic mercury by renal (proximal) tubular epithelial cells (Berndt *et al.,* 1985; Baggett and Berndt, 1986; deCeaurriz *et al.,* 1994; Tanaka *et al.,* 1990; Zalups, 1995, 1998a; Zalups and Barfuss, 1995a,b, 1998; Zalups and Lash, 1994, 1997b). Although it is not clear where these mercuric conjugates of GSH form in the body, it is reasonably certain that they do form and that they are involved in at least the luminal uptake of mercury along the proximal tubule. This conclusion is based on evidence for the direct involvement of the activity of the γ-glutamyltransferase in the renal tubular uptake of inorganic mercury (deCeaurriz *et al.,* 1994; Tanaka *et al.,* 1990; Zalups, 1995; Zalups and Lash, 1994). The primary function of this enzyme, which is localized almost exclusively in the luminal membrane of proximal tubular cells, is to cleave the γ-glutamylcysteine bond on molecules of GSH that are present in the tubular lumen. On the basis of the current findings, and those from two of our previous studies (Zalups and Barfuss, 1996; Zalups, 1998b), it appears that at least some of the molecules of GSH incorporated in these mercuric conjugates probably arise from the liver. The present data also indicate that GSH status within the renal tubular epithelial cells likely plays an important role in the renal tubular retention and accumulation of inorganic mercury.

The mechanisms involved in the renal tubular uptake and transport, retention, and accumulation of inorganic mercuric ions are complex. Uptake of inorganic mercury by proximal tubular epithelial cells in the kidney is dependent not only on the amount of inorganic mercury delivered to the site of uptake

(and accumulation), but also on the types of ligands bound to the mercuric ions that allow the inorganic mercury to be transported into the tubular epithelial cells (Zalups and Barfuss, 1995a,b, 1998). Another issue of which very little is known, is what happens to the mercuric ions once they are taken up into proximal tubular cells. It appears that thiols such as GSH are not only involved in the uptake of inorganic mercury by tubular epithelial cells, but that they are also involved in the intracellular disposition of inorganic mercury within renal tubular epithelial cells.

As was the case in our previous study (Zalups and Barfuss, 1996), biliary ligation caused the concentration and content of mercury in the blood to increase in rats. Other data from the present study indicate that biliary ligation also causes the burden of mercury in blood to increase in rats pretreated with BSO, DEM, or acivicin, even though the pretreatments themselves do not appear to have a significant effect on the disposition of mercury in the blood. In control rats or rats pretreated with BSO or DEM, but not acivicin, biliary ligation also caused the content of mercury in the liver to increase. These findings in the control rats confirm our previous hepatic dispositional findings that biliary ligation causes a significant increase in the hepatic burden of mercury (Zalups and Barfuss, 1996). The effects of biliary ligation on the disposition of mercury in the liver and blood in the present study are probably due to the limited ability of the liver to secrete inorganic mercury (presumably in the form of a conjugate of GSH [Ballatori and Clarkson, 1984]) into the bile, due to the biliary obstruction, which likely caused a back up of mercury in systemic circulation. Much like in the kidney, the changes detected in the hepatic disposition of mercury following biliary ligation do not appear to correlate well with the hepatic content of GSH, at least not during the initial hour after the administration of mercury.

The increased levels of mercury in the blood induced by biliary ligation are also likely the cause for the increased amounts of mercury that were detected in the tissues of the small intestine. With the exception of the animals pretreated with acivicin, biliary ligation caused the content of mercury in the tissues of the small intestine to increase. By contrast, biliary ligation did not have any significant effects on the disposition of mercury in the tissues of the large intestine.

Significant amounts of inorganic mercury were also detected in the luminal contents of both the small and large intestines of all of the rats that had undergone biliary ligation. We believe these findings serve as evidence for some form of secretion of mercury (from the blood into the lumen) within these two intestinal segments. Moreover, the present findings indicate that some forms of depletion of GSH cause significant decreases in this secretory-like movement of inorganic mercury into the lumen of these two intestinal segments. Since significant amounts of inorganic mercury were detected in the lumen of both the small and large intestine within a very short time after the injection of mercuric chloride, it seems unlikely that much of this mercury represents a pool of mercury in enterocytes that had sloughed off during the normal turnover of the intestinal epithelium. A more likely explanation is that inorganic mercury undergoes transepithelial transport into the lumen of the respective intestinal segments by enterocytes. Two additional mechanisms that may have contributed to the pool of mercury in the lumen of the small intestine include delivery of mercury via pancreatic secretions and intercellular leak of mercury through the leaky tight junctions of the enterocytes. Since the pancreatic duct was not ligated, it is also possible that some mercury was delivered into the lumen of the small intestine via pancreatic secretions. However, regardless of the precise mechanism involved, data from the animals whose bile duct had been ligated indicate the movement of mercuric ions occurs from the blood into the lumen of the small and large intestines and that this movement is influenced significantly by some forms of depletion of GSH. Evidence for intestinal secretion of inorganic mercury has also been provided in two other recent studies (Zalups and Barfuss, 1996; Zalups, 1998b).

In conclusion, we demonstrate that chemically induced GSH depletion and biliary ligation, either individually or in combination, alter significantly the accumulation of inorganic mercury in the kidneys, liver, and blood during the first hour after the injection of a nontoxic dose of mercuric chloride. In addition, biliary ligation combined with individual methods used to chemically deplete GSH in the present study have additive effects on the renal disposition of administered inorganic mercury. The present findings also confirm some form of secretory-like movement of mercuric ions from the blood into the lumen of the small and large intestines. In addition to confirming the existence of some form of intestinal secretion of mercury, the present findings indicate that thiol status influences this secretory-like process.

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