INTESTINAL HANDLING OF MERCURY IN THE RAT: IMPLICATIONS OF INTESTINAL SECRETION OF INORGANIC MERCURY FOLLOWING BILIARY LIGATION OR CANNULATION

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Three sets of experiments were carried out to determine if there is an intestinal secretory component in the fecal excretion of administered inorganic mercury. In the first set of experiments the disposition of a nontoxic 0.5-μmol/kg intravenous dose of inorganic mercury was evaluated in control rats and rats whose bile duct had been ligated. Data collected 24 h after the administration of mercuric chloride indicated that some inorganic mercury had moved from the blood across the epithelium into the lumen of the stomach, small intestine, and large intestine. This secretory movement of mercury was most prominent in the small intestine. Interestingly, the renal uptake and accumulation of mercury were diminished significantly in the rats whose bile duct had been ligated. A time-course experiment showed that the maximum amount of secretory movement of mercury into the lumen of the small intestine occurred during the initial 12 h after the injection of mercuric chloride. By the end of 24 h after the injection of mercuric chloride, much of the inorganic mercury secreted in the small intestine appeared to have moved down into the large intestine. In a third experiment, the disposition of mercury was evaluated in control rats and rats who had their bile duct cannulated. The rationale for this third experiment was to study the disposition of mercury under conditions where obstruction of biliary outflow from the liver would not be as much of an issue as with ligation of the bile duct. Evidence for movement of mercury into the lumen of the intestines was also obtained from the rats whose bile duct had been ligated. Eighteen hours after the injection of mercuric chloride the amount of mercury in the luminal compartment of the small intestine was not statistically different between the two groups of rats. Approximately 1.7–2.1% of the administered dose was present in the luminal contents of the small intestine. Decreased renal uptake of mercury was also detected in the rats whose bile duct had been cannulated. The findings from the present study show that when bile flow is obstructed or diverted, clear evidence for secretory movement of mercury into the lumen of the gastrointestinal (GI) tract can be demonstrated. These findings also indicate that the secretory movement of mercury into the lumen of the GI tract is a mechanism that contributes significantly to the pool of mercury that is excreted in the feces.

Exposure to various forms of mercury is becoming an increasing problem in our society, and very little is known about the mechanisms involved in the accumulation, toxicity, and excretion of mercury. This is especially true when it comes to the intestinal handling of inorganic forms of mercury.

Numerous sets of in vivo data from rats indicate that between 3 and 10% of a nontoxic dose of inorganic mercury is excreted in the feces during the first 24 h after exposure (Rothstein & Hayes,1960; Zalups 1995a, 1995b, 1995c, 1995d; Zalups & Barfuss, 1996; Zalups & Minor, 1995; Zalups et al., 1987). Until very recently (Zalups & Barfuss, 1996), it has been assumed that much of the fecal excretion of both inorganic and organic forms of mercury in humans and other mammals occurs primarily through a mechanism involving the hepatic secretion of mercury into the bile (Ballatori & Clarkson, 1984a, 1984b, 1985). Apparently, very little attention has been payed to the possibility of the intestines playing a significant role in contributing to the fecal elimination of mercury. There has been, however, some discussion of the potential role of the intestines in the enterohepatic recycling of the organic mercurial methylmercury (Ballatori & Clarkson, 1985; Clarkson et al., 1973). Much of this recycling is most likely due to the lipophilic properties of methylmercury, since evidence for enterohepatic recycling of inorganic forms of mercury is lacking.

The first line of evidence indicating that the intestines may be contributing to the fecal excretion of inorganic mercury was obtained fortuitously in one our recent studies in which the effects of preventing or diverting biliary outflow on the renal disposition of inorganic mercury were being evaluated (Zalups & Barfuss, 1996). In this study it was demonstrated that some level of fecal excretion of mercury occurred in animals whose bile duct had been ligated. Since the bile duct had been ligated in these animals, the only explanation for the fecal excretion of mercury is that there was some form of intestinal secretion of inorganic mercury. This was quite surprising, considering the current line of thinking on the mechanisms involved in the fecal excretion of mercury. Consequently, we put forth the hypothesis that the fecal excretion of inorganic mercury consists of two mechanisms: one mechanism involving the hepatic secretion of mercury into the biliary tree, and another mechanism, as of yet uncharacterized, that involves the movement of mercury from the blood across the intestinal epithelium into the lumen.

The aim of the present study was to provide additional evidence in support of this hypothesis and to better characterize the intestinal contribution to the fecal excretion of systemically administered inorganic mercury. In the present study, two sets of experiments were designed to evaluate the disposition of mercury in the tissues and luminal contents of the stomach, small intestine, and large intestine in rats whose bile duct had been ligated. In these experiments, the disposition of mercury in the liver, kidneys, and blood was also evaluated. In another set of experiments, the influence of diverting biliary outflow for 18 h into an indwelling abdominal reservoir on the intestinal, hepatic, renal, and hematologic disposition of mercury was evaluated. The findings from these experiments clearly indicate that one of the mechanisms involved in the fecal elimination of inorganic

mercury involves secretory-like movement of mercuric ions from blood into the lumen of the gastrointestinal (GI) tract.

MATERIALS AND METHODS

Animals, Groups, and Experiments

Male Sprague-Dawley rats weighing 175–200 g were used in the present investigation. Three separate experiments were carried out. Experiment 1 was designed to test the hypothesis that some of the inorganic mercury in blood is delivered into the lumen of the GI tract by some secretory-like mechanism in animals whose bile duct had been ligated. Experiment 2 was designed to evaluate (during the initial 24 h after the administration of a nontoxic dose of mercuric chloride) the time course for the enterohepatic disposition of mercury in animals whose bile duct had been ligated. Experiment 3 was designed to evaluate the enterohepatic disposition of mercury in rats whose bile duct had been cannulated. The rationale for this third experiment comes from the fact that the enterohepatic disposition of mercury could be studied under conditions where bile flow was not impeded significantly in conscious animals (in which a cannulated catheter drained bile into an indwelling reservoir placed in the abdomen).

All animals were housed individually in plastic cages before experimentation. In experiments 1 and 3, all animals were housed in plastic metabolic cages for the duration of the respective experiment. In experiment 1, two groups of rats were used. One group served as a control and the other group of rats underwent biliary ligation immediately prior to the intravenous injection of a nontoxic 0.5-µmol/kg dose of mercuric chloride. The use of a nontoxic dose of mercury was important in the present study, since the disposition of inorganic mercury could be studied in the absence of complicating factors associated with cellular injury in target organs. The disposition of mercury in kidneys, liver, blood, stomach, and intestines and the urinary and fecal excretion of mercury were determined 24 h after the injection of mercury. In experiment 2, three groups of rats were used. All of these rats underwent biliary ligation just prior to the intravenous injection of the 0.5-µmol/kg dose of mercuric chloride. At 1, 12, and 24 h after the injection of mercury, the disposition of mercury in the tissue and luminal compartments of the stomach, small intestine, and large intestine was evaluated. The disposition of mercury in the kidneys, liver, and blood was also evaluated.

In experiment 3, the disposition of mercury in the intestines, liver, kidneys, and blood and the urinary and fecal excretion of mercury were evaluated and compared in a group of control rats and a group of rats whose bile duct had been cannulated. These evaluations were performed 18 h after the animals had been given the intravenous nontoxic dose of

mercuric chloride. Biliary excretion of mercury was also evaluated in the animals whose bile duct had been cannulated.

Surgical Procedures

In experiments 1 and 2, all animals were anesthetized with a 50-mg/kg intraperitoneal dose of sodium pentobarbital. In the animals whose bile duct was to be ligated, a midline incision was made through the skin and abdominal muscles. Subsequently, the intestines were moved to the left side of the animal until the bile duct could be identified. Two 4-0 silk ligatures were tied securely around the midportion of the bile duct. In experiment 1, the proximal portion of the duodenum was also ligated. After the intestines were placed back into their proper position, the abdominal muscles were sewn together and the skin was approximated using 9-mm stainless-steel wound clips. All animals were allowed to recover from the anesthesia induced by sodium pentobarbital prior to beginning the respective experiment. This was done to minimize the potential effects of sodium pentobarbital on the renal accumulation of mercury. We have demonstrated that barbiturates can cause a significant reduction in the renal uptake of administered inorganic mercury while animals are unconscious (unpublished data).

In experiment 3, all animals were also anesthetized with a 50-mg/kg intraperitoneal dose of sodium pentobarbital. Once anesthesia was induced, a midline incision was made through the skin and abdominal muscles. After the intestines were moved to the left side of the animal, the bile duct was identified and cleared of fat and connective tissue. Then in the animals designated for biliary cannulation, a small incision was made in the bile duct (midway between its origin in the liver and its termination in the duodenum) and the beveled tip of a 7-cm-long piece of polyethylene (PE) 10 (ID 0.011 in, OD 0.024 in) tubing was inserted into the bile duct in a manner retrograde to the natural flow of bile. The tip of the polyethylene cannula was pushed up close to the point where the duct emanates from the liver. The cannula was secured in place with two sterile 4-0 silk ligatures. Once bile flow out of the opposite end of the cannula was established (generally within 5 min), the opposite end of the cannula was inserted into the mouth of a latex balloon and secured there with two silk ligatures. The cannula and attached balloon were placed into the abdominal cavity and then the abdominal muscles were sewn together with sterile 4-0 silk suture and the skin was approximated with sterile 9-mm wound clips. In the control animals, the intestines were moved in and out of the animals, and then the abdominal muscles were sewn together and the skin was approximated with 9-mm wound clips.

Injection of Mercuric Chloride

All the animals in experiments 1, 2, and 3 were administered a 0.5-µmol/kg dose of mercuric chloride ($HgCl_2$) into the right femoral vein

approximately 1 h after the animals had recovered from anesthesia induced by sodium pentobarbital (used to perform surgeries). The 0.5-μmol/kg dose of $HgCl_2$ was administered while the animals were anesthetized lightly with ether. Radioactive inorganic mercury in the form of mercuric chloride ($^{203}HgCl_2$; Buffalo Materials Corp., Buffalo, NY) was added to the injection solution containing the cold mercuric chloride. The specific activity of $^{203}Hg^{2+}$ used in the present study ranged between 20 and 25 mCi/mg. Each injection solution was designed to deliver 0.5 μmol Hg^{2+}/kg and 4 μCi/kg in 2.0 ml (0.2 ml injection volume per 100 g body weight) 0.9% (w/v) aqueous sodium chloride.

Protocol for Femoral Injections

The technique used to administer injections into the femoral vein is the same as that outlined previously (Zalups, 1993). While each animal was anesthetized as described earlier, a small incision was made through the skin in the midventral region of the right thigh with a pair of small surgical scissors to expose the femoral vein and artery. After the fascia around the femoral vein was trimmed, the appropriate volume of injection solution was injected into the vein. Immediately after the injection, the site of injection was swabbed with cotton gauze and the opposite ends of the incised skin were approximated with sterile 9-mm stainless-steel wound clips.

Collection of Urine and Feces

Urine and feces were collected for 24 h in experiment 1 and for 18 h in experiment 3. These samples were obtained from each animal after it was placed individually in a plastic metabolic cage following the injection of inorganic mercury.

Acquisition and Handling of Tissue

At the end of each experimental period (following the injection of the 0.5-μmol/kg dose of inorganic mercury) in experiments 1, 2, or 3, a sample of blood was obtained from the inferior vena cava of each animal. All animals were first anesthetized with a 75-mg/kg dose of sodium pentobarbital (ip) just prior to obtaining this sample of blood. One milliliter of blood was placed and sealed in a preweighed 12 × 75 mm polystyrene round-bottom gamma-counting tube. The other 1 ml of blood was spun down for 10 min 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 obtaining the sample of blood, the kidneys and liver were excised, both cleared of fat and connective tissue, and weighed quickly. Two kidneys from each animal were cut in half along the transverse plane. One half of each kidney was placed and sealed in a preweighed polystyrene gamma-counting tube. In experiment 1, a 3-mm section of kidney was sliced away from the mid-

region of the remaining half of the left kidney and samples of the cortex, outer and inner stripes of the outer medulla, and inner medulla were obtained. A 1-g sample of liver was also obtained. The stomach and small and large intestine were also excised from each animal. The luminal contents of these segments of the gastrointestinal tract were removed and collected with multiple washes of 0.9% aqueous sodium chloride using a 5-ml syringe. All samples of renal and hepatic tissues and each excised segment of the intestinal tract and its corresponding luminal contents were placed and sealed individually in preweighed gamma-counting tubes. The bile collected from each of the rats whose bile ducts had been cannulated in experiment 3 was also placed individually into preweighed counting tubes.

At the end of experiment 1 and 3, the total amount of urine excreted by each animal in 24 and 18 h, respectively, was determined gravimetrically and a 1-ml sample was placed and sealed in a preweighed gamma-counting tube. The entire amount of feces excreted by each animal in 24 and 18 h, respectively, was divided up and placed in multiple (if needed) 16×100 mm polypropylene tubes.

Determinations of the Content of Mercury in Tissues, Organs, Bile, Urine, and Feces

The amount of radioactivity in the samples of tissues, organs, bile, urine, feces, and injection solutions (standards) was determined by counting the samples in a 1282 Compugamma CS deep-well gamma spectrometer with a 3-in sodium iodide crystal (Wallac, Gaithersburg, MD) operating at a counting efficiency of approximately 50% for $^{203}Hg^{2+}$. The content of mercury in each sample was calculated by dividing the activity (dpm) in the sample by the specific activity (dpm/nmol) of the corresponding injection solution. Concentrations of mercury in samples of tissues and organs were expressed as a percent of the administered dose per gram of wet tissue. Total contents of mercury in the kidneys, liver, blood, segments of the intestinal tract, luminal contents of the segments of the intestinal tract, and the total amount of bile excreted were expressed simply as a percent of the administered dose. The total volume of blood in the rat was assumed to be approximately 6% of body weight. Excretion of mercury in the urine or feces is expressed as a percent of the administered dose excreted in 24 or 18 h.

Statistical Analysis

Values are expressed as mean ± SE. Differences between corresponding means for each set of data for each part of experiment 1 and 3 were evaluated statistically using Student's unpaired t-test for independent samples. In experiment 2, differences between corresponding means were evaluated statistically using a one-way analysis of variance followed by Tukey's post hoc multiple comparison procedure. Since per-

cent scores are not normally distributed, data expressed as a percent of a total were first normalized using the arcsine transformation prior to performing any statistical analysis. The arcsine transformation takes the arcsine of the square root of the decimal fraction of the percent score. The level of significance for all statistical analyses was chosen a priori to be $p < .05$.

RESULTS

Experiment 1

There was no significant difference in body weight between the two groups animals used in this experiment (Table 1).

Effects of Biliary Ligation of the Disposition of Mercury in the Gastrointestinal Tract Twenty-four hours after the injection of the non-toxic 0.5-μmol/kg dose of mercuric chloride, the amount of mercury present in both the tissue and luminal compartments of the stomach was significantly greater in the animals whose bile duct had been ligated than in the control animals (Figure 1). The most prominent effects of biliary ligation were detected in the small intestine. As in the stomach, the amount of mercury in the tissue and luminal compartments of the small intestine was significantly greater in the rats whose bile duct had been ligated than in the control rats. By contrast, the amount of mercury in the luminal contents of the large intestine was significantly less in the rats whose bile duct had been ligated than in the control rats. There was no significant difference in the amount of mercury in the tissue component of the large intestine between the two groups of rats.

Effects of Biliary Ligation on the Disposition of Mercury in the Kidneys, Liver, and Blood In the rats whose bile duct had been ligated, the amount of mercury in the total renal mass was approximately 28%

TABLE 1. Concentration of injected Hg^{2+} in renal zones and blood of control rats and rats whose bile duct had been ligated

Group	Animal body weight (g)	$[Hg^{2+}]$ in renal cortex (% dose/g)	$[Hg^{2+}]$ in OS/OM (% dose/g)	$[Hg^{2+}]$ in IS/OM (% dose/g)	$[Hg^{2+}]$ in renal inner medulla (% dose/g)	$[Hg^{2+}]$ in blood (% dose/g)
Control (n = 5)	215 ± 2	38.3 ± 1.23	23.4 ± 1.20	2.31 ± 0.95	1.57 ± 0.18	0.14 ± 0.00
Ligated bile duct (n = 5)	213 ± 2	27.3 ± 1.59[a]	11.2 ± 11.2[a]	1.21 ± 0.09	1.18 ± 0.23	0.41 ± 0.04[a]

Note. Values represent mean ± SE for five rats. OS/OM, renal outer stripe of the outer medulla. IS/OM, renal inner stripe of outer medulla. All animals received an intravenous 0.5-μmol/kg dose of mercuric chloride containing 4 μCi/kg $^{203}Hg^{2+}$ after biliary ligation. The disposition of inorganic mercury in the renal tissues and blood was evaluated 24 h after injection of the mercuric chloride.
[a]Significantly different ($p < .05$) from the corresponding mean for the control group of rats.
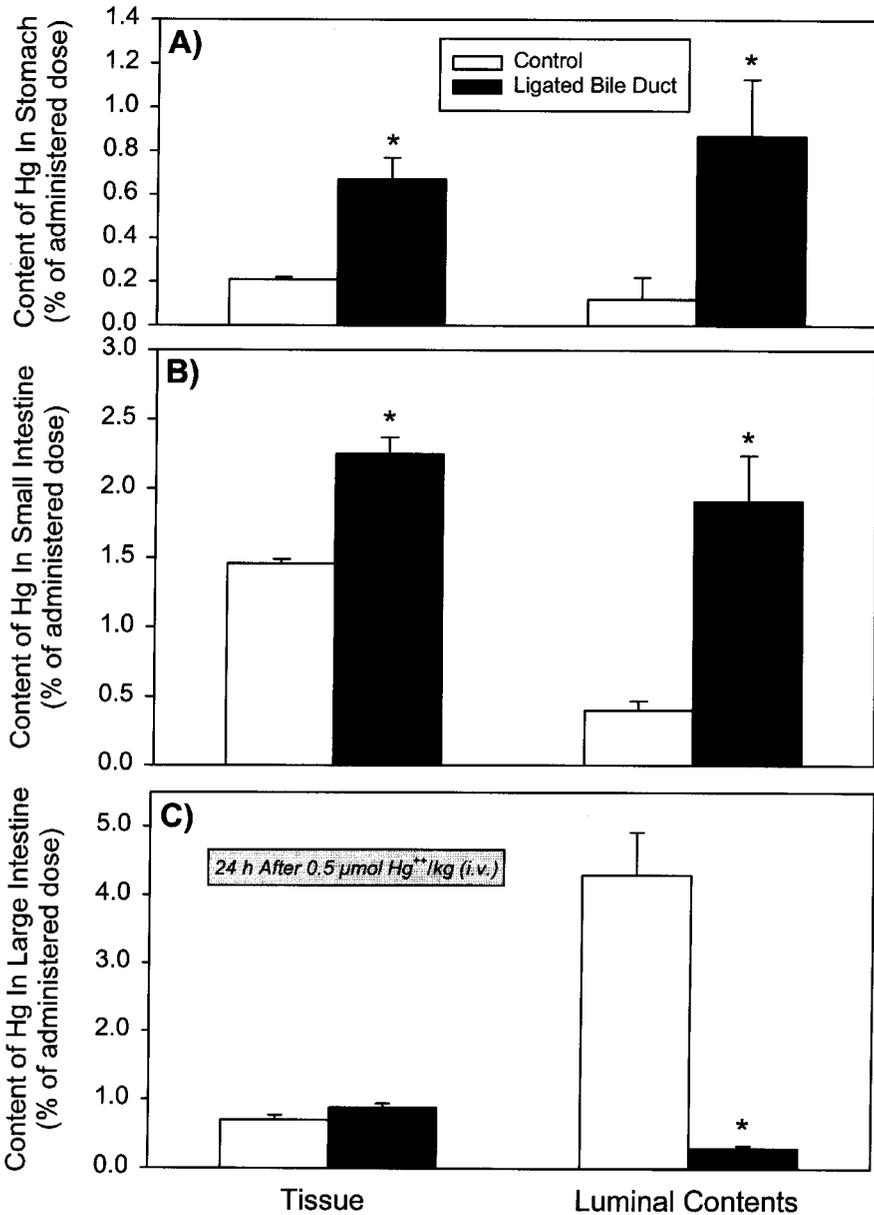
FIGURE 1. Content of inorganic mercury (% dose) in the tissue and luminal compartments of the (A) stomach, (B) small intestine, and (C) large intestine of control rats and rats whose bile duct had been ligated. All animals received a single 0.5-μmol/kg nontoxic intravenous dose of mercuric chloride 24 h prior to the evaluation of the disposition of mercury. Each value represents a mean ± SE for five animals. Asterisk indicates significantly different ($p < .05$) from the corresponding mean for the control group of rats.

less than that in the control rats (Figure 2). This decrease in the renal bur-
den of mercury in the animals whose bile duct had been ligated was due
to a relative decrease in the uptake and/or accumulation of mercury in
both the renal cortex and outer stripe of the outer medulla (Table 1).

Biliary ligation caused the accumulation of mercury in both the liver
and blood to increase markedly. In the rats whose bile duct had been lig-
ated, the 24-h accumulation of mercury in the liver and blood was
approximately 100% and 140% greater, respectively, than that in the con-
trol rats (Figure 2).

**Effects of Biliary Ligation on the Urinary and Fecal Excretion of
Mercury**   About 5.5% of the administered dose was excreted in the
urine by the control rats, while only a little more than 1% of the dose was
excreted in the urine by the rats whose bile duct had been ligated (Figure
3). There was no excretion of feces in the rats whose bile duct had been
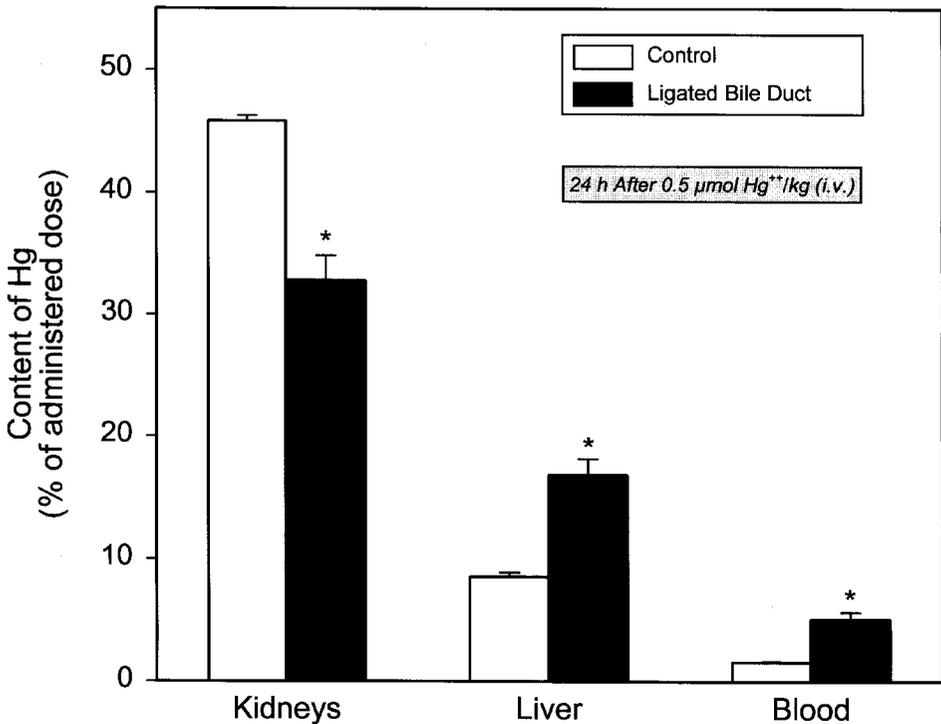ligated, and thus, there was no fecal excretion of mercury. The control

FIGURE 2. Content of inorganic mercury (% dose) in the kidneys, liver, and blood of control rats and
rats whose bile duct had been ligated. All animals received a single 0.5-μmol/kg nontoxic intra-
venous dose of mercuric chloride 24 h prior to the evaluation of the disposition of mercury. Each
value represents a mean ± SE for five animals. Asterisk indicates significantly different ($p < .05$) from
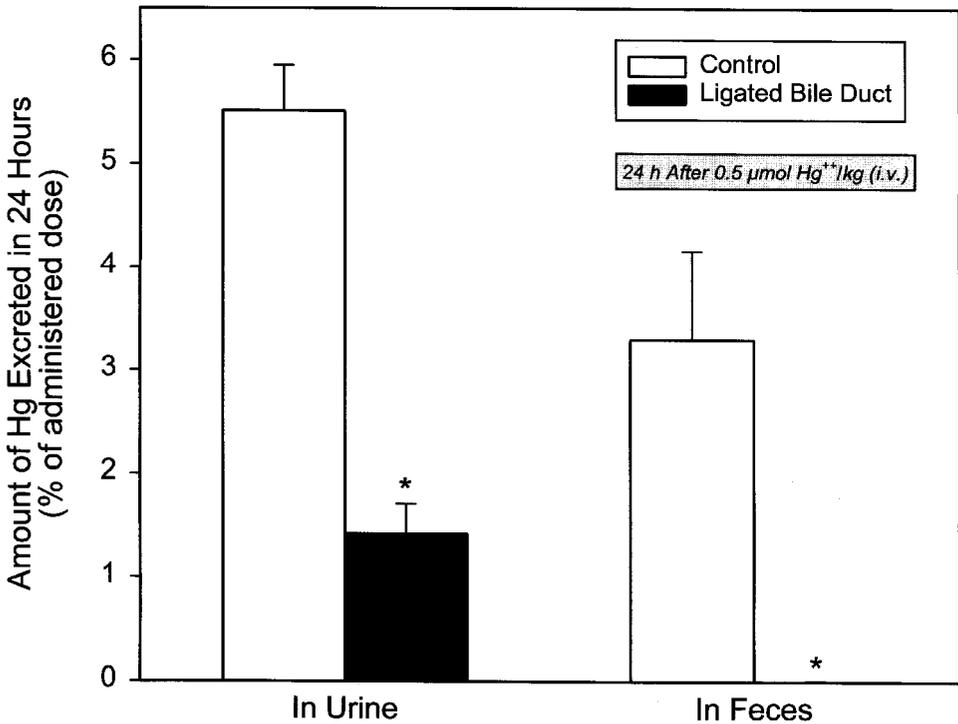the corresponding mean for the control group of rats.

FIGURE 3. Amount of mercury excreted (% dose) in the urine and feces in 24 h by control rats and rats whose bile duct had been ligated. All animals received a single 0.5-μmol/kg nontoxic intravenous dose of mercuric chloride 24 h prior to the evaluation of the disposition of mercury. Each value represents a mean ± SE for five animals. Asterisk indicates significantly different ($p < .05$) from the corresponding mean for the control group of rats.

rats, however, excreted approximately 3–4% of the administered dose of mercury in the feces during the 24 h of the experiment.

Experiment 2

No statistically significant changes in body weight were detected amongst the three groups of rats used in this experiment (Table 2).

Time Course for Changes in the Gastrointestinal Disposition of Mercury   At 1 h after the intravenous injection of the nontoxic 0.5-μmol/kg dose of mercuric chloride, slightly less than 0.4% of the administered dose of mercury was present in the tissue of the stomach and less than 0.1% of the dose was present in the luminal contents of the stomach (Figure 4). In the rats studied at 12 and 24 h after the injection of mercury, the amount of mercury in the tissues of the stomach was less than 0.3% of the dose. This amount was significantly less than that measured at 1 h after the injection of mercury. No statistically significant changes in the amount of mercury present in the luminal contents of the stomach were detected during the 24 h of this experiment.

Significant changes were detected in the disposition of mercury in the small intestines (Figure 4). At 1 h after the injection of mercuric chloride, about 2.8% of the dose of mercury was present in the tissue component of the small intestine and about 0.7% of the dose was present in the luminal contents of the small intestine. In the rats studied at 12 h after the administration of mercuric chloride, the content of mercury in the tissue and luminal compartments was significantly less and greater, respectively, than that in the animals studied at 1 h. The amount of mercury in the luminal contents of the small intestine of these animals was almost twice that in the animals studied at 1 h. In the animals studied 24 h after the injection of mercuric chloride, the amount of mercury present in the luminal contents of the small intestine was significantly less than that measured at 12 h. The amount of mercury in the tissue component of the small intestine in the rats studied at 24 h was not significantly greater than that in the rats studied at 12 h, but was significantly greater than that in the rats studied at 1 h.

No significant difference in the amount of mercury in either the tissue or luminal compartment of the large intestine was detected between the rats studied at 1 and 12 h after the injection of mercuric chloride (Figure 4). However, in the rats studied 24 h after the injection of mercuric chloride, there was a great increase in the luminal content of mercury in the large intestine relative to that in the other two groups of rats. Moreover, the amount of mercury in the tissue compartment of the large intestine was significantly less than that in the rats studied at either 1 or 12 h after the injection of mercuric chloride.

When the tissue components or the luminal components of the stomach, small intestine, and large intestine were combined, an interesting profile emerged (Figure 5). Between 1 and 24 h, the amount of mercury

TABLE 2. Concentration of injected Hg^{2+} in renal zones and blood of rats whose bile duct had been ligated

Group	Animal body weight (g)	$[Hg^{2+}]$ in renal cortex (% dose/g)	$[Hg^{2+}]$ in OS/OM (% dose/g)	$[Hg^{2+}]$ in IS/OM (% dose/g)	$[Hg^{2+}]$ in renal inner medulla (% dose/g)	$[Hg^{2+}]$ in blood (% dose/g)
1-h (n = 5)	202 ± 4	23.0 ± 1.62	8.65 ± 0.75	1.32 ± 0.10	1.73 ± 0.18	1.78 ± 0.11
12-h (n = 5)	201 ± 3	28.4 ± 1.71[a]	10.5 ± 1.16	0.99 ± 0.06[a]	1.06 ± 0.15[a]	0.46 ± 0.02[a]
24-h (n = 4)	193 ± 3	27.1 ± 2.11[a]	13.3 ± 1.38[a]	1.27 ± 0.11	1.38 ± 0.13	0.26 ± 0.01[a]

Note. Values represent mean ± SE for five rats. OS/OM, renal outer stripe of the outer medulla. IS/OM, renal inner stripe of outer medulla. All animals received an intravenous 0.5-μmol/kg dose of mercuric chloride containing 4 μCi/kg $^{203}Hg^{2+}$ after biliary ligation. The disposition of inorganic mercury in the renal tissues and blood was evaluated 1, 12, or 24 h after injection of the mercuric chloride.

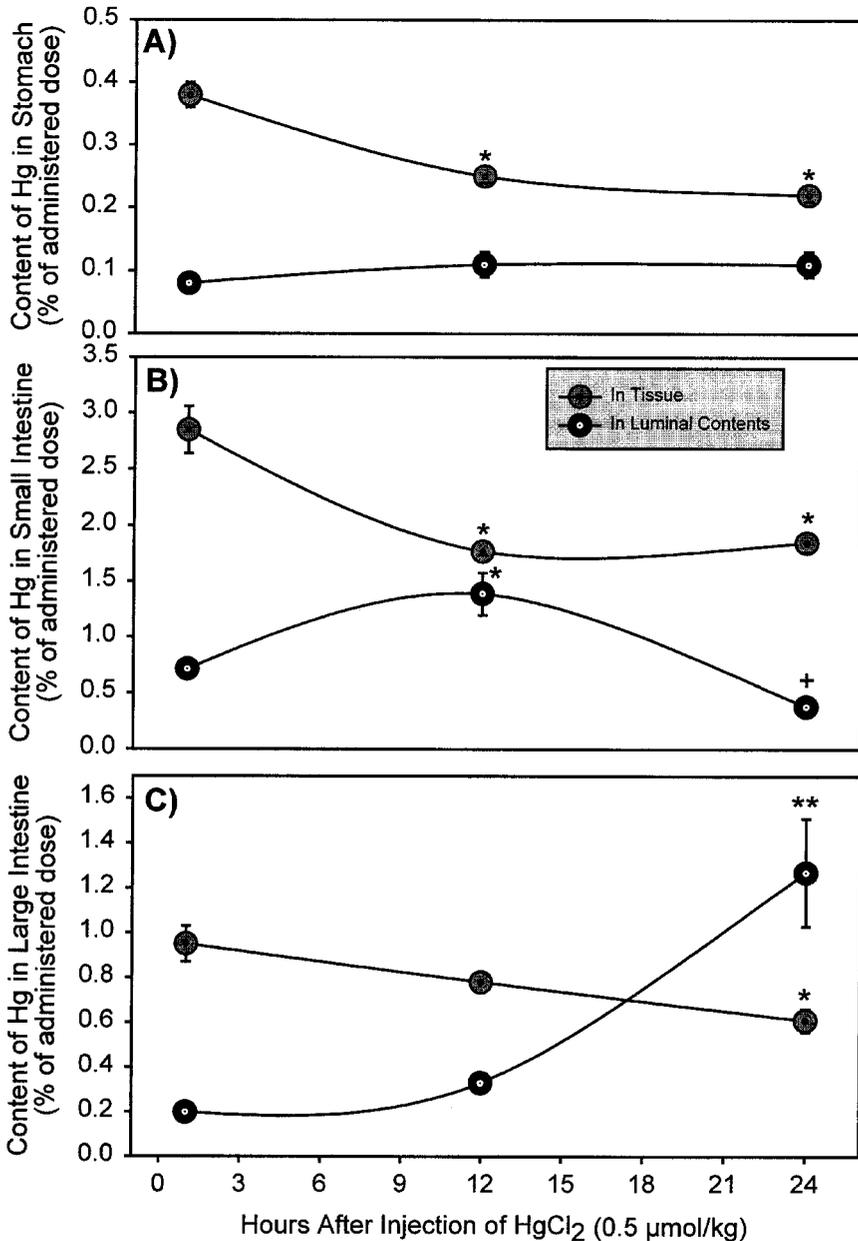[a]Significantly different ($p < .05$) from the corresponding mean for the 1-h group of rats.

FIGURE 4. Content of inorganic mercury (% dose) in the tissue and luminal compartments of the (A) stomach, (B) small intestine, and (C) large intestine at 1, 12, or 24 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride in rats whose bile duct had been ligated. Each value represents a mean ± SE for four to five animals. Asterisk indicates significantly different ($p < .05$) from the corresponding mean for the group of rats evaluated 1 h after the injection of mercuric chloride. Double asterisk indicates significantly different ($p < .05$) from the corresponding mean for the groups of rats evaluated 1 h or 12 h after the injection of mercuric chloride. Plus sign indicates significantly different ($p < .05$) from the corresponding mean for the group of rats evaluated 12 h after the injection of mercuric chloride.

in the tissue compartment decreases and the amount of mercury in the luminal compartment increases.

Time Course for Changes in the Disposition of Mercury in the Kidneys, Liver, and Blood In the rats studied 1 h after the injection of mercuric chloride, approximately 23% of the administered dose was in the total renal mass, 22% of the dose was in the blood, and approximately 16% of the dose was present in the liver (Figure 6). The renal burden of mercury in the rats studied 12 h after the injection of mercuric chloride was approximately 32% of the dose and the amount of mercury in the blood was only slightly greater than 5% of the dose. The differences in the burden of mercury in the kidneys and blood between the rats studied at 1 and 12 h were significantly different. Although the hepatic content of mercury in the rats studied at 12 h was approximately 20% of the administered dose, there was not a statistically significant difference in the hepatic burden of mercury between the animals studied at 1 and 12 h.
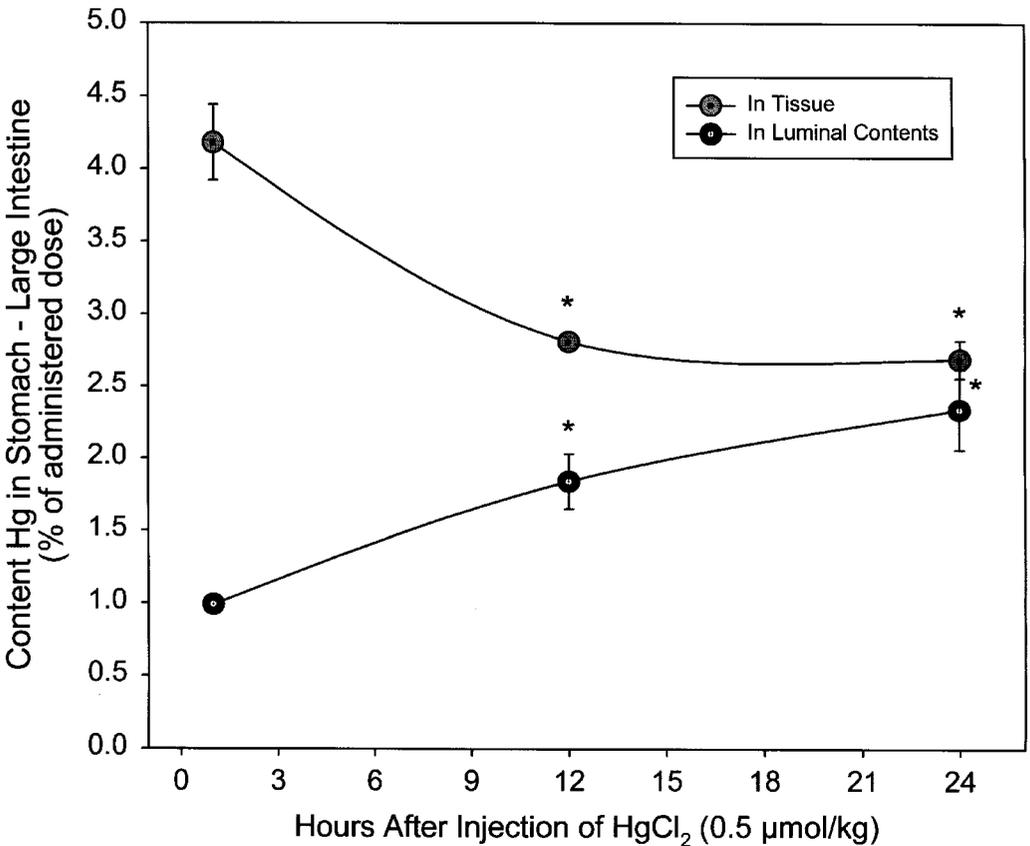

FIGURE 5. Content of inorganic mercury (% dose) in the tissue and luminal compartments of the stomach + small intestine + large intestine at 1, 12, or 24 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride in rats whose bile duct had been ligated. Each value represents a mean ± SE for four to five animals. Asterisk indicates significantly different ($p < .05$) from the corresponding mean for the group of rats evaluated 1 h after the injection of mercuric chloride.
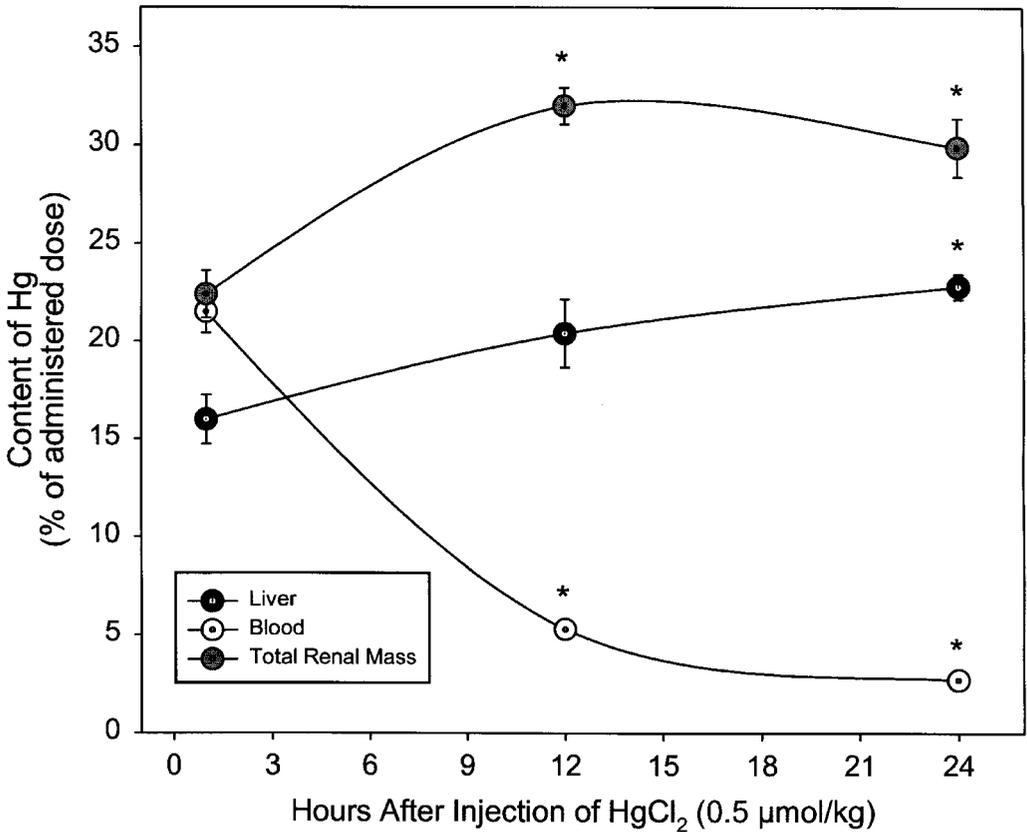
FIGURE 6. Content of inorganic mercury (% dose) in the kidneys, liver, and blood at 1, 12, or 24 h after the intravenous injection of a 0.5-µmol/kg dose of mercuric chloride in rats whose bile duct had been ligated. Each value represents a mean ± SE for four to five animals. Asterisk indicates significantly different ($p < .05$) from the corresponding mean for the group of rats evaluated 1 h after the injection of mercuric chloride.

The total amount of mercury in the kidneys, liver, and blood in the rats studied at 24 h was approximately 30%, 23%, and 3% of the administered dose, respectively. The differences in the burden of mercury in the kidneys, liver, and blood between the animals studied at 1 and 24 h were significant. However, the differences in these same parameters between the animals studied at 12 and 24 h were not statistically significant.

The time-dependent changes in the renal burden of mercury were due primarily to changes in the accumulation of mercury in the renal cortex and outer stripe of the outer medulla (Table 2). There were time-dependent changes in the disposition of mercury in the inner stripe of the outer medulla and inner medulla; however, these were not as great as those detected in the other two zones of the kidney.

Experiment 3

No significant difference in body weight was detected between the two groups of rats used in this experiment (Table 3).

Influence of Biliary Cannulation on the Disposition of Mercury in the Gastrointestinal Tract By the end of 18 h after the injection of the non-toxic 0.5-μmol/kg dose of mercuric chloride, the amount of mercury in the tissue and luminal compartments of the stomach was significantly greater in the rats whose bile duct had been cannulated than in the control rats (Figure 7).

In animals whose bile duct was cannulated, there was also significantly more mercury in the tissue compartment of the small intestine than the control rats. Interestingly, both groups of rats had about 1.7–2.1% of the dose in the luminal compartment of the small intestine (Figure 7).

There was about 0.7–0.8% of the dose in the tissue compartment of the large intestine in both groups of rats. There was, however, significantly more mercury in the luminal compartment of the large intestine in the control rats than in the rats whose bile duct had been cannulated. About 2.5% of the dose was in the luminal compartment of the control rats, while only about 0.6% of the dose was present in the same compartment of the rats whose bile duct had been cannulated.

Influence of Biliary Cannulation on the Disposition of Mercury in the Kidneys, Liver, and Blood Eighteen hours after the injection of mercuric chloride, the renal burden of mercury was significantly lower in the rats whose bile duct had been cannulated than in the control rats (Figure 8). By contrast, the amounts of mercury in the liver and blood were both sig-

TABLE 3. Dispositional data from control rats and rats whose bile flow was diverted into an intra-abdominal balloon reservoir

Group	Animal body weight (g)	$[Hg^{2+}]$ in blood (% dose/g)	Amount of Hg^{2+} excreted in the bile (% dose/18 h)
Control (n = 5)	191 ± 1	0.18 ± 0.01	NA
Cannulated bile duct (n = 6)	176 ± 3	0.31 ± 0.04[a]	0.58 ± 0.10

Note. Values represent mean ± SE for five rats. All animals received an intravenous 0.5-μmol/kg dose of mercuric chloride containing 4 μCi/kg $^{203}Hg^{2+}$ after biliary cannulation. The disposition of inorganic mercury in blood and bile was evaluated 18 h after injection of the mercuric chloride. NA, not applicable.

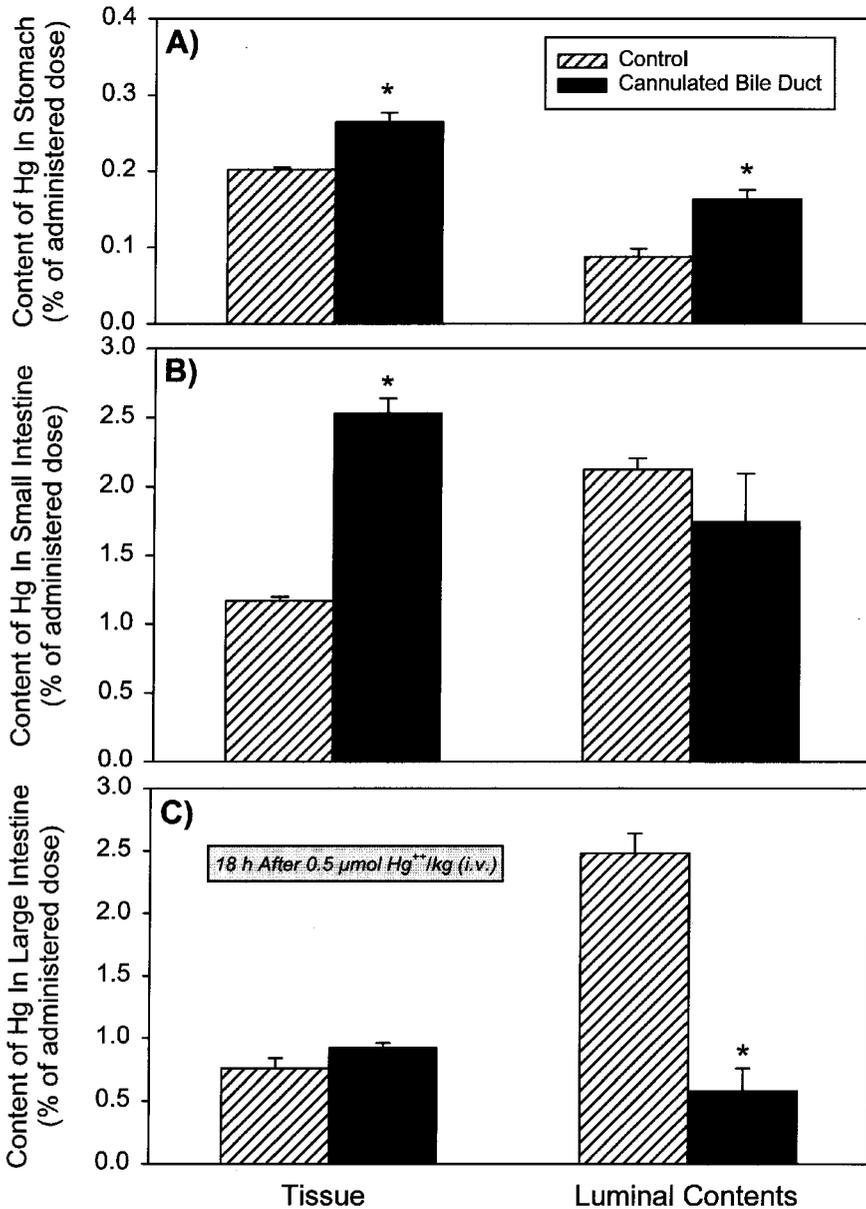[a]Significantly different ($p < .05$) from the corresponding mean for the control group of rats.

FIGURE 7. Content of inorganic mercury (% dose) in the tissue and luminal compartments of the (A) stomach, (B) small intestine, and (C) large intestine at 18 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride in control rats and rats whose bile duct had been cannulated. In the rats whose bile duct had been cannulated, the cannula was connected to an indwelling latex reservoir placed in the abdominal cavity. Each value represents a mean ± SE for five to six animals. Asterisk indicates significantly different ($p < .05$) from the corresponding mean for the control group.
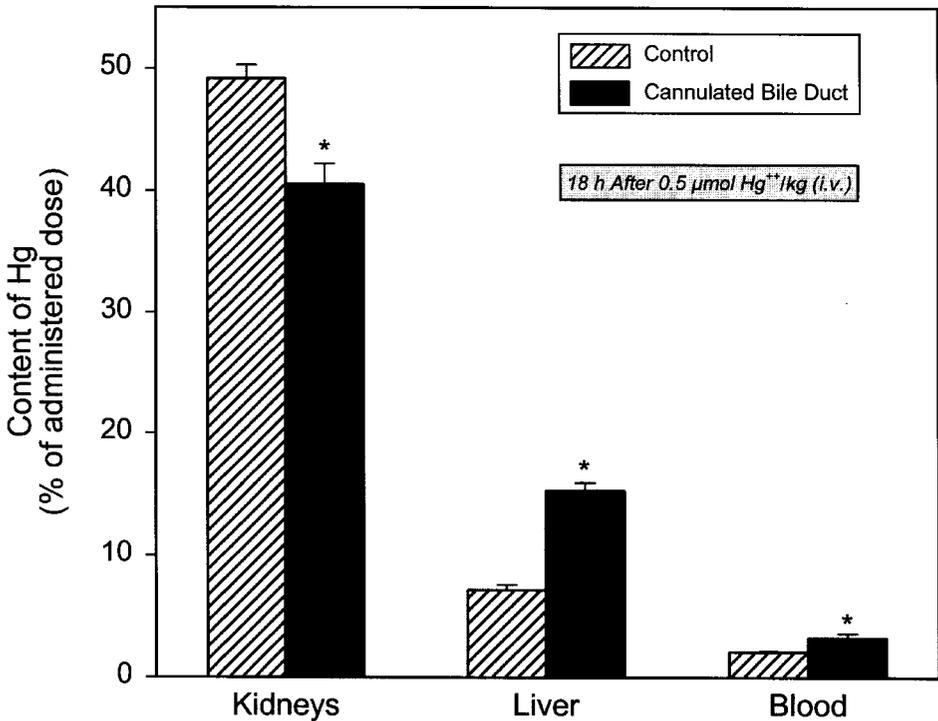
FIGURE 8. Content of inorganic mercury (% dose) in the kidneys, liver, and blood at 18 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride in control rats and rats whose bile duct had been cannulated. In the rats whose bile duct had been cannulated, the cannula was connected to an indwelling latex reservoir placed in the abdominal cavity. Each value represents a mean ± SE for five to six animals. Asterisk indicates significantly different ($p < .05$) from the corresponding mean for the control group.

nificantly greater in the rats whose bile duct had been cannulated than in the control rats.

The animals whose bile duct had been cannulated excreted an average of 7.4 ± 0.4 ml of bile during the 18 h of the experiment. An average of only 0.58% of the dose of administered mercury was eliminated in this volume of bile (Table 3).

Influence of Biliary Cannulation on the Urinary and Fecal Excretion of Mercury   Approximately half as much mercury was excreted in the urine in 18 h by the rats whose bile duct had been cannulated than by the control rats. The control rats excreted a little over 4% of the dose in the urine in 18 h, while the experimental group of rats excreted slightly less than 2% of the dose in the same period of time (Figure 9).

The control rats excreted about 4% of the dose in the feces during the 18 h of the experiment. By contrast, the fecal excretion of mercury in the
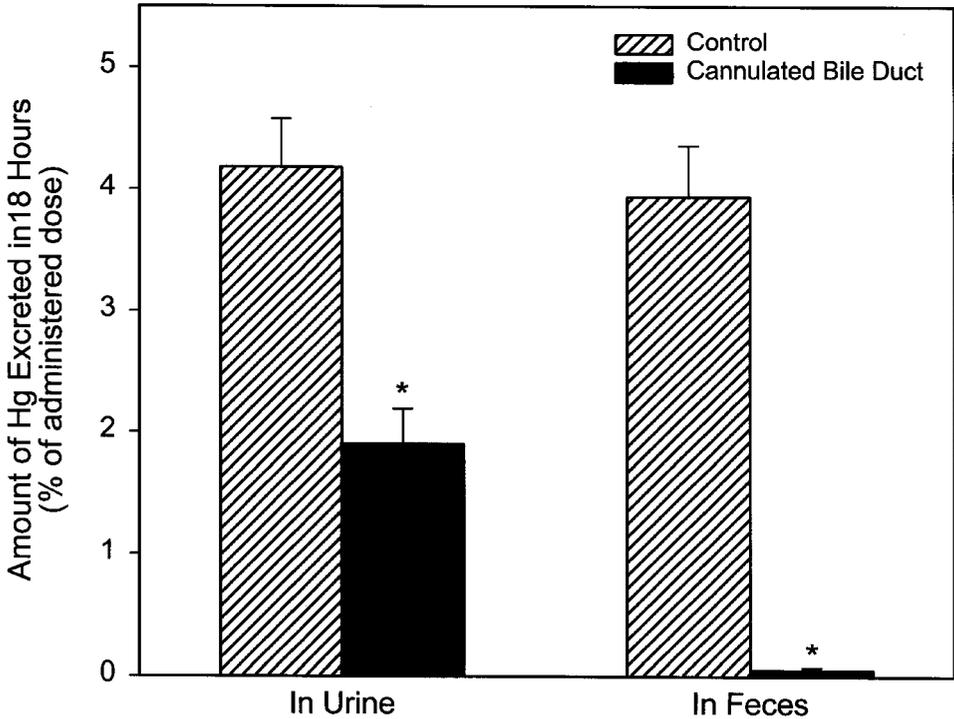
FIGURE 9. Amount of inorganic mercury (% dose) excreted in the urine and feces during the initial 18 h following the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride in control rats and rats whose bile duct had been cannulated. In the rats whose bile duct had been cannulated, the cannula was connected to an indwelling latex reservoir placed in the abdominal cavity. Each value represents a mean ± SE for five to six animals. Asterisk indicates significantly different ($p < .05$) from the corresponding mean for the control group.

rats whose bile duct had been cannulated was only around 0.1% of the dose in 18 h, largely due to a decreased rate of excretion of feces.

DISCUSSION

Data collected from animals whose bile duct had been ligated prior to receiving a nontoxic dose of inorganic mercury show clearly that there is some form of secretory-like movement of inorganic mercury from the blood into the lumen of the gastrointestinal (GI) tract. The predominant site where this secretion occurs is in the small intestine, although some inorganic mercury also appears to move in a secretory-like manner into the lumen of the stomach and large intestine. On the basis of the data obtained from experiment 2, it appears that the majority of the movement of mercury into the luminal compartment of the small intestine occurs during the initial 12 h after the injection of mercuric chloride. During this time the content of mercury in the tissue compartment decreases while the

content of mercury in the luminal compartment increases. By the end of the first 24 h after the injection of inorganic mercury, most of the mercury that is in the lumen of the small intestine appears to have moved down into the large intestine, presumably by peristalsis.

It is very likely that some level of secretory-like movement of mercury into the lumen of the GI tract also occurs in normal rats exposed to mercury, inasmuch as evidence for secretory-like movement of mercury into the small intestine was also obtained from rats whose bile duct had been cannulated (in which obstruction of bile flow was not as significant a concern). The current data indicate that the percent of the administered dose of mercury in the luminal compartment of the small intestine of the rats whose bile duct had been cannulated is not statistically different from that in control animals, indicating a rather significant level of secretory movement of mercury into the lumen of the small intestine of the rats whose bile duct had been cannulated.

It should be mentioned that the term "secretion" is used loosely in the context of the phenomenon being discussed in this article inasmuch as the precise mechanism(s) by which mercury enters into the lumen of the GI tract are not clear. Although it is possible that some of the movement of mercury into the lumen of the GI tract may be due to sloughing off of epithelial cells as a part of the normal turnover of the epithelium, the dispositional findings obtained 1 h after the injection of inorganic mercury in the rats whose bile duct had been cannulated strongly lead one to believe that there is likely some type of actual transepithelial transport of mercury. There is a rather convincing body of current evidence indicating that mercury can be taken up from the blood by proximal tubular epithelial cells in the kidney. More specifically, this evidence indicates that some thiol (presumably glutathione or cysteine) conjugate of mercury is transported across the basolateral membrane by the organic anion transporter (Zalups, 1995a; Zalups & Minor, 1995). It is not clear at present whether actual transepithelial secretion of mercury occurs along segments of the mammalian nephron. On the basis of these findings on the transport of mercury in the kidney, it seems probable that inorganic mercuric conjugates of cysteine and/or glutathione might be taken up by the basolateral membrane of enterocytes, which are also transporting epithelial cells.

Although over 90% of the inorganic mercury in the plasma of blood is bound to albumin and other large proteins (Zalups & Lash, 1994), there is a significant pool of both cysteine and glutathione in the plasma to which mercuric ions can bind. Inorganic mercuric ions have a very high affinity for sulfhydryl groups, and cysteine and glutathione are the most abundant small thiol-containing compounds in blood. Both cysteine and glutathione are in the plasma of the rat at a concentration of approximately 10 μM (Lash & Jones, 1985).

Fecal excretion of mercury is one of the predominate means by which mercury is eliminated from the body (Zalups & Lash, 1994). Until very

recently, it had been assumed by many that the primary, if not only, mechanism by which mercury entered into the GI tract following parenteral administration of a mercuric compound was by the biliary secretion of mercury. The findings from the present study clearly indicate that there is at least one other major component involved in the fecal excretion of inorganic mercury, and perhaps other forms of mercury as well. Regardless of mechanism(s), the current findings indicate that the movement of mercury from the blood into the lumen of the GI tract (mainly in the small intestine) contributes significantly to the pool of mercury eliminated through fecal excretion. The exact degree to which intestinal secretion of mercury contributes to the fecal excretion of inorganic mercury is not known at present, but on the basis of the relatively small amount of mercury excreted in the bile in the rats whose bile duct had been cannulated, it appears to be a rather substantial contribution.

Obstruction or diversion of bile flow also caused the renal uptake and/or accumulation of mercury to be decreased significantly. This is consistent with the preliminary findings from a recent study in which the renal disposition of mercury was evaluated in rats treated similarly (Zalups & Barfuss, 1996). It was speculated that this decrease might be due to preventing an enteric reabsorptive recycling of cysteine conjugates of mercury. There are data indicating that both inorganic and organic forms of mercury form conjugates with glutathione in hepatocytes prior to being secreted into the bile (Ballatori & Clarkson, 1984a, 1984b, 1985). However, the amount of inorganic mercury excreted in the bile (in animals whose bile duct had been cannulated) cannot account for the amount of mercury that was not taken up by the kidneys during the period of time in which bile was being collected. Thus, it appears that the decreased renal uptake of mercury that occurs in association with the obstruction or diversion of bile flow is due to some other mechanism. Since substantial amounts of glutathione are secreted into the bile (which then gets degraded to cysteine by the γ-glutamyltransferase and dipeptidase found on the luminal surface of the biliary canaliculi and small-intestinal enterocytes), it is logical to surmise that diminished reabsorption of cysteine occurs in the small intestine following obstruction or diversion of biliary outflow, and that this causes an alteration in plasma thiol status. By reducing plasma concentrations of cysteine and/or glutathione, there would be a decrease in the amount of mercuric–thiol conjugates being formed in the plasma, which would affect the rate of delivery of mercury–thiol conjugates to the luminal and/or basolateral plasma membranes of the epithelial cells lining the proximal tubule. There are preliminary data from my laboratory supporting this notion (Zalups & Lash, 1997). There are additional data indicating that as little as 1 h after ligation of the bile duct, there is a significant decrease in the concentration of both glutathione and cysteine in the plasma of blood (unpublished findings). Further work is under way to confirm these findings.

It is not exactly clear what mechanism(s) is/are responsible for the increased hepatic accumulation of mercury in the rats that had their bile duct either ligated or cannulated. A highly probable explanation relates to the putative decreases in plasma concentrations of cysteine and glutathione induced by the biliary ligation or cannulation. Diminished levels of cysteine (especially when prolonged) could lead to depletion of glutathione and general thiol status in hepatocytes, inasmuch as cysteine is the rate-limiting substrate in the synthesis of glutathione. Depletion of glutathione in hepatocytes, in turn, would presumably diminish the probability for formation of mercuric conjugates of glutathione, which would seemingly diminish the ability of the hepatocyte to secrete mercury into the bile. Recent evidence indicates that when glutathione in the liver is depleted, the hepatic accumulation of injected inorganic mercury increases (Zalups & Lash, 1997), which is consistent with the current hypothesis. Another contributing factor to the increased hepatic content of mercury induced by biliary ligation or cannulation is the diminished urinary excretion of mercury that probably caused the increased concentration of mercury in the blood. Enhanced concentrations of mercury in the blood would generate an electrochemical gradient for mercury, favoring its movement into hepatocytes.

In summary, the primary findings from this present study provide clear evidence for the movement of administered inorganic mercury into the lumen of the GI tract by some type of secretory mechanism(s). These findings are quite significant, since the current views on the fecal elimination of mercury have focused almost exclusively on the biliary secretion of mercury. The current findings indicate that at least two mechanisms are involved, one of them being biliary secretion of mercury and the other being intestinal secretion of mercury. With further research, it is hoped that the precise mechanism(s) that govern the fecal elimination of this important environmental toxicant will be discovered. The present findings also confirm the fact that some aspect or component of the renal uptake and accumulation of mercury is linked to the hepato-biliary-enteric systems.

REFERENCES

Ballatori N., and Clarkson, T. W. 1984a. Inorganic mercury secretion into bile as a low molecular weight complex. *Biochem. Pharmacol.* 33:1087–1092.

Ballatori, N., and Clarkson, T. W. 1984b. Dependence of biliary secretion of inorganic mercury on the biliary transport of glutathione. *Biochem. Pharmacol.* 33:1093–1098.

Ballatori, N., and Clarkson, T. W. 1985. Biliary secretion of glutathione and of glutathione-metal complexes. *Fundam. Appl. Toxicol.* 5:816–831.

Clarkson, T. W., Small, H., and Norseth, T. 1973. Excretion and absorption of methly mercury after poly-thiol resin treatment. *Arch. Environ. Health* 26:173–176.

Lash, L. H., and Jones, D. P. 1985. Distribution of oxidized and reduced forms of glutathione and cysteine in rat plasma. *Arch. Biochem. Biophys.* 240:583–592.

Rothstein, A., and Hayes, A. D. 1960. The metabolism of mercury in the rat studied by isotope techniques. *J. Pharmacol. Exp. Ther.* 130:166–176.

Zalups, R. K. 1993. Influence of 2,3-dimercaptopropane-1-sulfonate (DMPS) and *meso*-2,3-dimercaptosuccinic acid (DMSA) on renal disposition of mercury in normal and uninephrectomized rats exposed to inorganic mercury. *J. Pharmacol. Exp. Ther.* 267:791–800.

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

Zalups, R. K. 1995b. Progressive losses of renal mass and the renal and hepatic disposition of administered inorganic mercury. *Toxicol. Appl. Pharmacol.* 130:121–131.

Zalups, R. K. 1995c. Accumulation and handling of inorganic mercury in the kidney after coadministration with glutathione. *J. Toxicol. Environ. Health* 44:385–399.

Zalups, R. K. 1995d. Renal disposition of mercury in rats after intravenous injection of inorganic mercury and cysteine. *J. Toxicol. Environ. Health* 44:401–413.

Zalups, R. K., and Barfuss, D. W. 1996. Diversion or prevention of biliary outflow from the liver diminishes the renal uptake of injected inorganic mercury. *Drug Metab. Dispos.* 24:480–486.

Zalups, R. K., and Lash, L. H. 1994. Advances in understanding the renal transport and toxicity of mercury. *J. Toxicol. Environ. Health* 42:1–44.

Zalups, R. K., and Lash, L. H. 1997. Depletion of glutathione in the kidney and the renal disposition of administered inorganic mercury. *Drug Metab. Dispos.* 25:516–523.

Zalups, R. K., and Minor, K. H. 1995. Luminal and basolateral mechanisms involved in the renal tubular uptake of inorganic mercury. *J. Toxicol. Environ. Health* 46:73–100.

Zalups, R. K., Klotzbach, J. M., and Diamond, G. L. 1987. Enhanced accumulation of inorganic mercury in renal outer medulla after unilateral nephrectomy. *Toxicol. Appl. Pharmacol.* 89:226–236.