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SIMULTANEOUS COEXPOSURE TO INORGANIC MERCURY AND CADMIUM: A STUDY OF THE RENAL AND HEPATIC DISPOSITION OF MERCURY AND CADMIUM

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This study was designed to evaluate the effects of simultaneous coexposure to inorganic mercury and cadmium on the renal and hepatic disposition of each metal. Dispositional changes were assessed in rats 1 h and 24 h after the coexposure to relatively low doses of the metals (which individually are nonnephrotoxic in rats). The rational for studying mercury and cadmium is that both of these metals are encountered frequently in the same contaminated areas. Coadministration of a 0.5-μmol/kg dose of mercuric chloride with a 10-μmol/kg dose of cadmium chloride resulted in a decrease in the net renal accumulation of inorganic mercury at 1 and 24 h after exposure. Assessment of the disposition of both metals in renal zones indicates that the decreased renal accumulation of inorganic mercury was due specifically to changes in the accumulation of mercury in the renal cortex. Coexposure to inorganic mercury and cadmium also caused both the hepatic accumulation of mercury and the urinary excretion of mercury to increase during the initial 24 h after coexposure. During the initial 1 h after coexposure, the content of mercury in the blood was enhanced significantly. However, by the end of the first 24 h after exposure, the content of mercury in the blood was lower than that in animals treated with only inorganic mercury, likely due to the increased urinary excretion of mercury. Interestingly, with the exception of decreased fecal excretion of cadmium, no other changes in the disposition of cadmium were detected in the animals treated with both mercury and cadmium. These novel findings indicate that at the doses of inorganic mercury and cadmium used in the present study, cadmium has profound effects on the renal and hepatic handling of mercury. Based on the present findings, it appears that cadmium [by some currently unknown mechanism(s)] interferes with the luminal and/or basolateral uptake and/or net accumulation of mercury along S_1 and S_2 segments of the proximal tubules, which results in an overall decrease in the renal burden of mercury and an increased rate in the urinary excretion of mercury.

Findings from numerous studies have provided a wealth of information about the manner in which toxic metals, such as mercury and cadmium, are taken up, processed, transported, and eliminated by cells in target organs, such as in the kidneys and liver (Clarkson, 1993; Klaassen & Liu, 1997; Prozialeck, 2000; Waalkes, 2000; Zalups & Lash, 1994; Zalups, 2000a, 2000b). By studying the transport and disposition of a metal, we

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have gained knowledge about the complex nature by which some metals exert their toxic effects. Although these types of studies are extremely important, and need to be continued, they provide little information about the interactions between specific metals in living organisms following coexposure or simultaneous exposure to two or more of these metals.

More often than not, exposure of humans to toxicants in environmental and/or occupational settings is complex, generally involving multiple toxicants and multiple factors. For example, many toxic waste sites contain numerous toxic metals, such as mercury, cadmium, lead and arsenic. Despite the tremendous amount of research dedicated to understanding the toxic effects of a single toxic metal, very little is known about how one metal may affect or influence the disposition, handling, and toxicity of another metal under conditions where there is simultaneous exposure to the metals (Madden & Fowler, 2000).

One of the principal aims of the present study is to begin assessing the effects of simultaneous coexposure to inorganic mercury and cadmium on the renal and hepatic disposition of each metal. The rationale for studying mercury and cadmium is that these two metals are encountered frequently in the same contaminated areas.

The findings from the present study represent ostensibly the first of their kind in which determinations of the simultaneous dispositions of both inorganic mercury and cadmium were made after coexposure to the metals. More specifically and importantly, the findings from this study indicate that simultaneous coexposure to cadmium and inorganic mercury does in deed have substantial effects on the disposition of each of the metals in target organs.

MATERIALS AND METHODS

General Experimental Design

The principal aim of the present study was to test the hypothesis that a low, nonnephrotoxic dose of cadmium, when coadministered with a low, nontoxic dose of inorganic mercury, affects significantly the disposition of mercury in the kidneys and liver. Two sets of experiments were carried out to test this hypothesis. In the first experiment (experiment 1), the disposition (levels) of mercury and/or cadmium (in the kidneys, liver, and blood) was evaluated 1 h after the intravenous coinjection of a 0.5-μmol/kg dose of inorganic mercury and/or a 10-μmol/kg dose of cadmium. Dispositional changes were assessed 1 h after treatment because maximal rates of renal and hepatic accumulation of inorganic mercury and cadmium occur during this initial period after treatment (as determined in our laboratories). In the second experiment (experiment 2), the disposition of inorganic mercury and/or cadmium (including the urinary and fecal excretion of mercury and cadmium) was assessed 24 h after treatment. The rationale for choosing this time to study dispositional changes is that near-maximal levels of the metals

are present in the kidneys and liver by the end of the initial 24 h after exposure.

Animals and Groups Used in Experiment

Three groups of four to five randomly selected male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN; each rat weighing between 200 and 240 g) were used in both experiments 1 and 2. Upon arriving from the vendor, the animals were allowed to acclimate to their new surroundings for several days. During this acclimation period, the animals were allowed food and water ad libitum. One of the groups served as the primary experimental group while the other two groups served as reference groups. In each experiment, the animals in the primary experimental group received intravenously, and simultaneously, a 0.5-μmol/kg dose of mercuric chloride and a 10-μmol/kg dose of cadmium chloride. One of the reference groups of rats received the 0.5-μmol/kg dose of mercuric chloride, while the other group of rats received the 10-μmol/kg dose of cadmium chloride.

Injections

The 0.5-μmol/kg dose of mercuric chloride and/or the 10-μmol/kg dose of cadmium chloride were administered into the right femoral vein after the animal had been anesthetized with ether. The dose of mercuric chloride and/or cadmium chloride was administered in 2.0 ml/kg normal saline. Radioactive inorganic mercury ($^{203}Hg^{2+}$, specific activity = 8.0 mCi/mg, Buffalo Materials Corp., Buffalo, NY) was added to all injection solutions containing mercuric chloride and radioactive cadmium ($^{109}Cd^{2+}$, specific activity = 1.55 mCi/mg, New England Nuclear, Boston) was added to all injection solutions containing cadmium chloride. The injections solutions were designed to deliver approximately 1.0 μCi $^{203}Hg^{2+}$ per animal and/or 3.0 μCi $^{109}Cd^{2+}$ per animal. This made it possible to study the disposition of inorganic mercury and/or cadmium using standard isotopic methods for gamma-emitting isotopes. The actual volume of injection solution delivered to each animal was determined both volumetrically and gravimetrically, to check for consistency. After the injection had been administered, the skin over the right femoral vein was approximated with sterile wound clips. Experiment 1 was terminated 1 h after treatment. Experiment 2 was terminated 24 h after treatment. This allowed for the assessment of effects on the urinary and fecal excretion of inorganic mercury and/or cadmium. At the termination of experiment 1 and experiment 2, all of the animals used were anesthetized with a 100-mg/kg dose of sodium pentobarbital, and the acquisition of organs and tissues was carried out in order to determine the disposition of inorganic mercury and/or cadmium.

Acquisition and Handling of Tissues and Organs

At the termination of each experiment, a 2.0-ml sample of blood was obtained from the inferior vena cava. One milliliter of blood was placed

and sealed in a preweighed 12 × 75 mm polystyrene round-bottom tube for gamma counting. The other 1 ml was spun down for 10 min at 20,000 × g to separate the cellular fraction of blood from the plasma. Both plasma and cellular fractions were placed individually, and sealed, in counting tubes. After the 2.0-ml sample of blood was obtained, the kidneys and liver were excised and were quickly cleared of fat and connective tissue and weighed. Each of the two kidneys from every animal was cut in half along the transverse plain. One half of each kidney was placed and sealed in a preweighed polystyrene counting tube. A 3-mm section of the remaining half of the left kidney was obtained. From this section of tissue, samples of cortex, outer and inner stripes of the outer medulla, and inner medulla were obtained and were placed in preweighed counting tubes. A 1-g section of liver was also obtained, placed, and sealed in a preweighed counting tube. The total volume of blood in each animal was estimated to be 6% of body weight.

Determinations of the Content of Mercury in Tissues and Organs

The amount of radioactivity in the samples of tissues, organs, urine, feces, and injection solution (standards) was determined by counting the samples in a 1282 Compugamma CS deep-well gamma spectrometer with a 3-in sodium iodide crystal (Wallac, Gaithersburg, MD) operating at a counting efficiency of approximately 50% for ^{203}Hg and about 45% for ^{109}Cd. 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 injection solution. Concentrations of mercury and/or cadmium in samples of tissue were expressed as a percent of the administered dose per gram tissue, while the content of mercury and/or cadmium in the kidneys, liver, and blood was expressed simply as a percent of the administered dose.

Statistical Analysis

All values presented are mean ± SE. All data expressed as a percent were first normalized using the arcsine transformation prior to applying parametric statistical analysis. This transformation takes the arcsine of the square root of the decimal fraction of the percent score.

In experiments 1 and 2, evaluation of differences between any two means was carried out by applying the two-tailed, unpaired, Student's *t*-test. The level of significance for all statistical analyses was chosen a priori to be $p < .05$.

RESULTS

Experiment 1

Renal Content of Mercury In the control group that received the 0.5-μmol/kg dose of inorganic mercury, approximately 43% of the dose of mercury was in the total renal mass 1 h after treatment (Figure 1). In contrast,

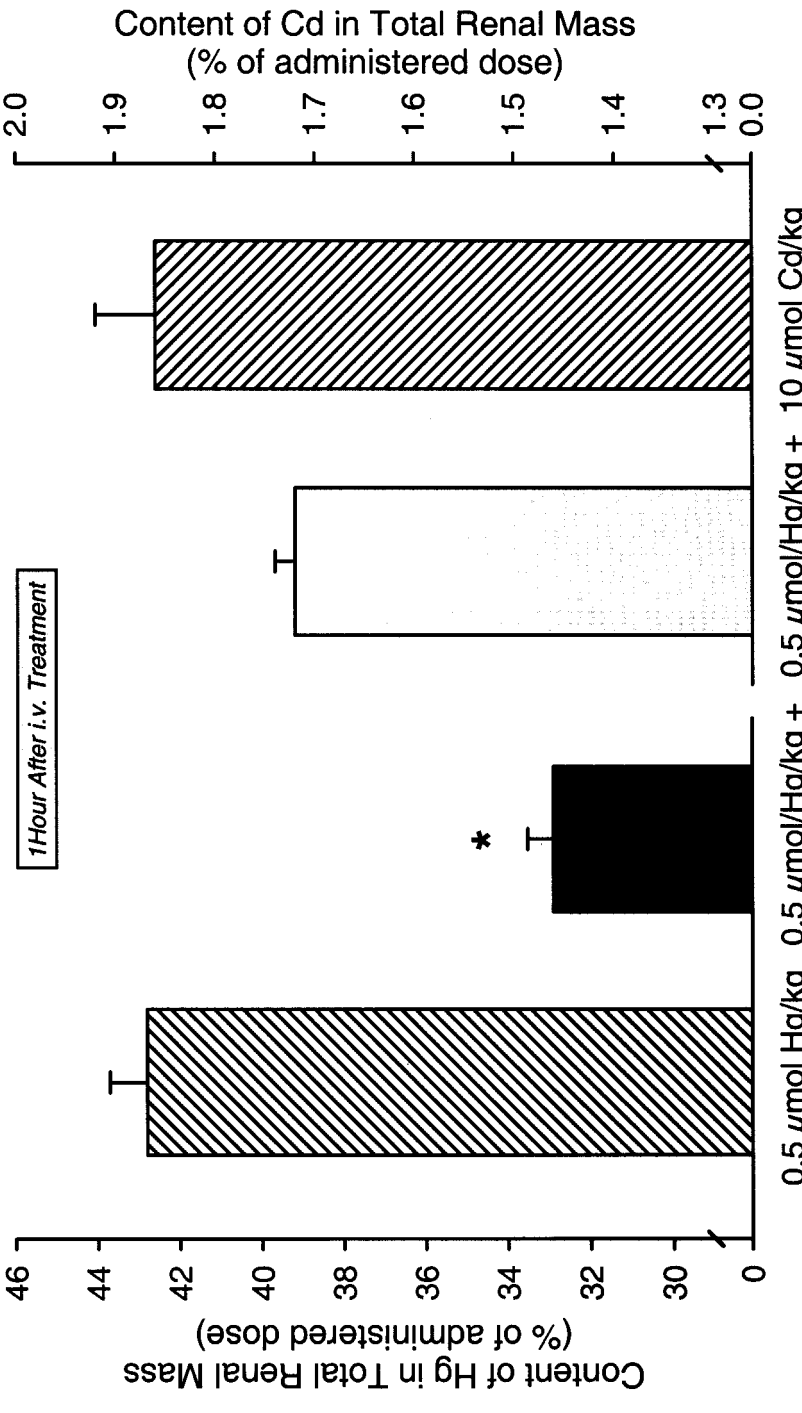

FIGURE 1. Content of inorganic mercury and/or cadmium (percent of the administered dose) in the total renal mass of rats 1 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride and/or a 10-μmol/kg dose of cadmium chloride. All values are expressed as the mean ± SE for four to five animals per group. Asterisk indicates significantly different from the corresponding mean for the group of rats treated only with the 0.5-μmol/kg dose of mercuric chloride.

only about 33% of the dose of mercury was in the total renal mass in the corresponding experimental animals treated simultaneously with the 0.5-μmol/kg dose of inorganic mercury and the 10-μmol/kg dose of cadmium chloride.

Renal Content of Cadmium Approximately 1.85% of the administered dose of cadmium was in the total renal mass of the second group of reference rats 1 h after treatment with the 10-μmol/kg dose of cadmium chloride (Figure 1). There was no statistically significant difference in the renal burden of cadmium between the experimental group treated with both inorganic mercury and cadmium and the reference group treated with just cadmium.

Concentration of Mercury in Renal Zones The average concentration of mercury in the renal cortex was approximately 27% greater in the animals injected only with inorganic mercury than that in the animals injected with both inorganic mercury and cadmium (Figure 2A). Moreover, the mean concentration of mercury in the renal outer stripe of the outer medulla was 25% greater in the animals injected only with inorganic mercury than that in the animals injected with both inorganic mercury and cadmium (Figure 2B).

Concentration of Cadmium in Renal Zones In the animals injected with inorganic mercury and cadmium, the mean concentration of cadmium in the renal cortex was about 14% lower than that in the animals injected only with cadmium. However, the difference in the renal cortical concentration of cadmium between the two groups proved not to be statistically significant. The concentration of cadmium in the renal outer stripe of the outer medulla was similar in the two corresponding groups that received the 10-μmol/kg dose of cadmium (Figure 2B).

Hepatic Content of Mercury Hepatic content of mercury was approximately 33% less in the animals treated with both inorganic mercury and cadmium than in the animals treated only with inorganic mercury (Figure 3).

Hepatic Content of Cadmium There was no significant difference in the hepatic content of cadmium at 1 h after treatment between the 2 corresponding groups treated with cadmium chloride (Figure 3).

Content of Mercury in Blood At 1 h after treatment, approximately 11.3% of the administered dose of mercury was present in the total blood volume of the rats injected only with inorganic mercury (Figure 4A). By contrast, 55% more mercury was present in the total blood volume of the rats injected with both inorganic mercury and cadmium.

Distribution of Mercury in Blood Of the mercury present in the blood of the rats treated only with inorganic mercury, approximately 14.7% was present in the plasma (Figure 4B). By comparison, about 25% less mercury was present in the plasma of the rats coinjected with inorganic mercury and cadmium.

Content and Distribution of Cadmium in the Blood No significant differences in the content of cadmium in the blood or the distribution of cadmium in the blood were detected between the group coinjected with inor-

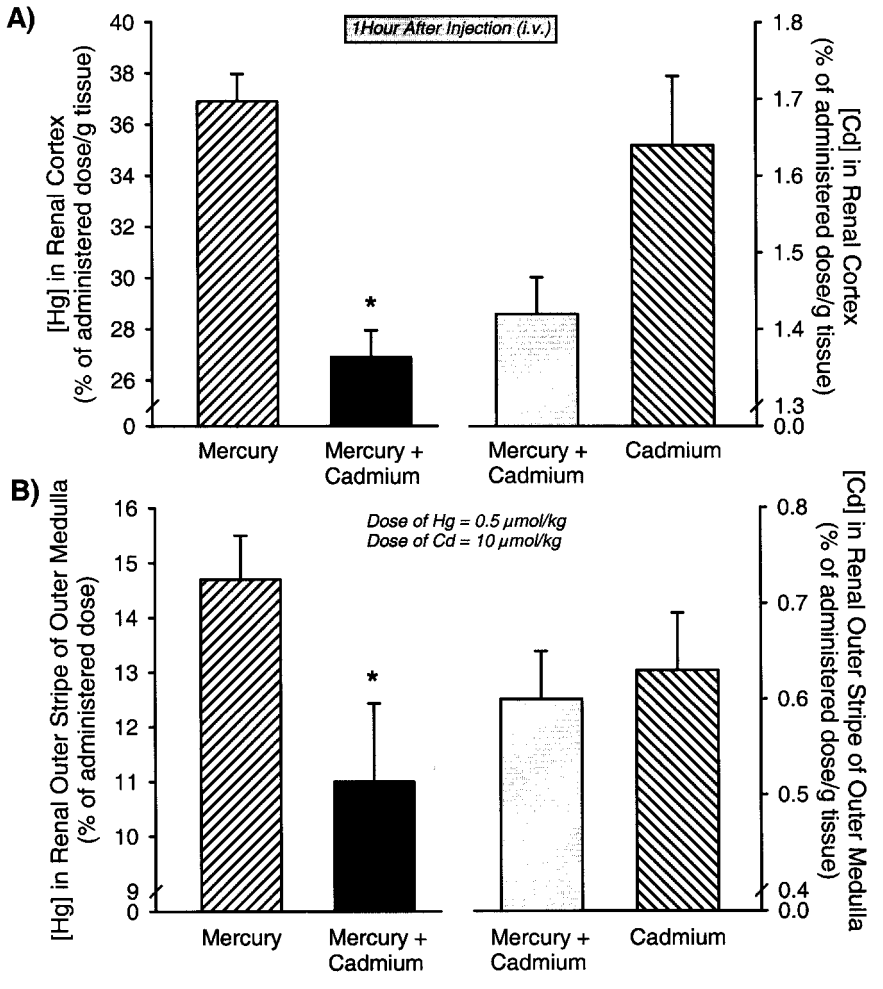

FIGURE 2. Concentration of inorganic mercury and/or cadmium (percent of the administered dose/gram tissue) in the renal cortex (A) and outer stripe of the outer medulla (B) of rats 1 h after the intravenous injection of a 0.5-µmol/kg dose of mercuric chloride and/or a 10-µmol/kg dose of cadmium chloride. All values are expressed as the mean ± SE for four to five animals per group. Asterisk indicates significantly different for the corresponding mean for the group of rats treated only with the 0.5-µmol/kg dose of mercuric chloride.

ganic mercury and cadmium and the group injected with only cadmium (Figure 4, A and B).

Experiment 2

Renal Content of Mercury At 24 h after treatment, approximately 48.5% of the administered dose of mercury was present in the total renal mass of the reference group of rats treated only with the 0.5-µmol/kg dose of mercuric chloride (Figure 5). By contrast, only about 41.5% of the ad-

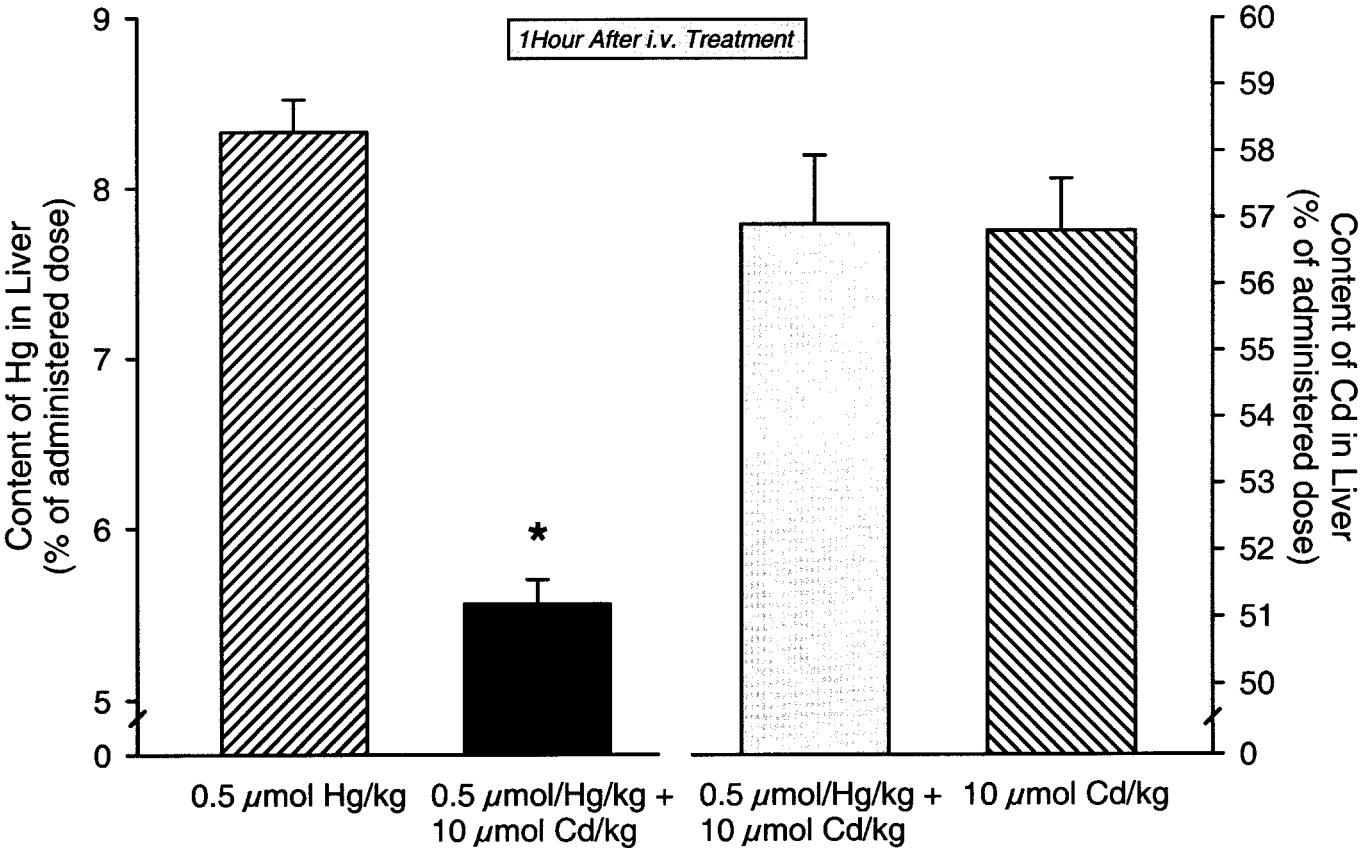

FIGURE 3. Content of inorganic mercury and/or cadmium (percent of the administered dose) in the liver of rats 1 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride and/or a 10-μmol/kg dose of cadmium chloride. All values are expressed as the mean ± SE for four to five animals per group. Asterisk indicates significantly different from the corresponding mean for the group of rats treated only with the 0.5-μmol/kg dose of mercuric chloride.

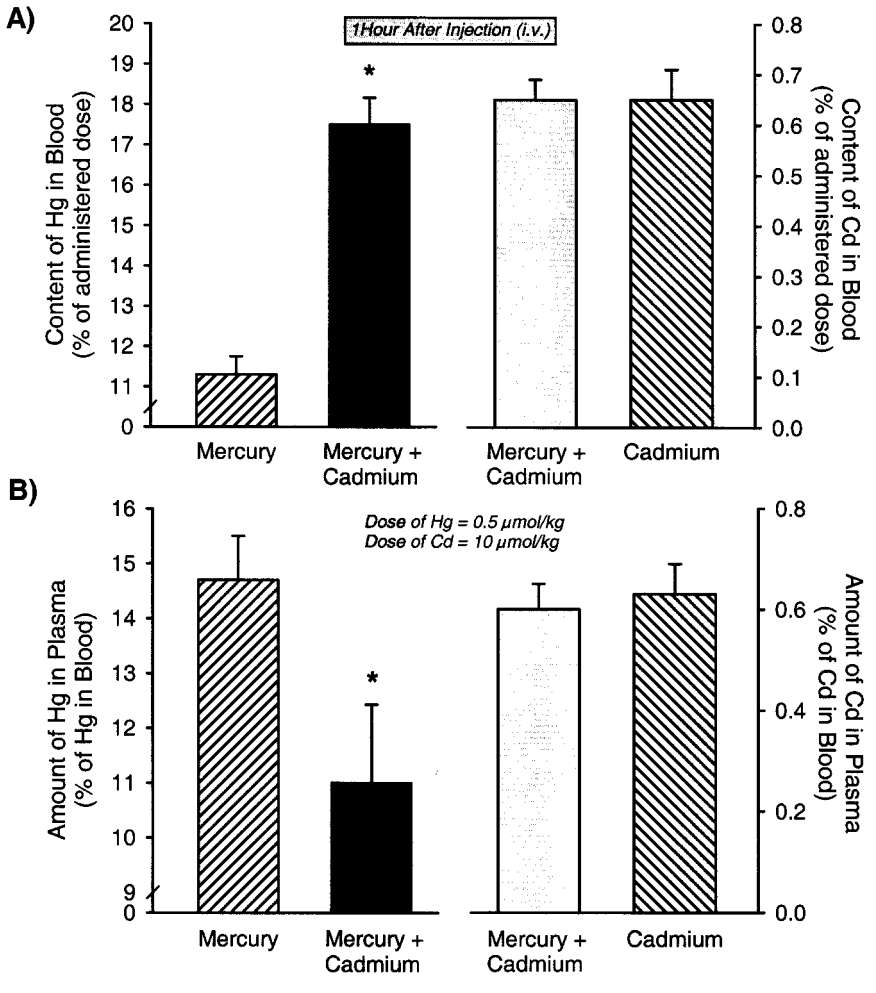

FIGURE 4. Content or amount of inorganic mercury and/or cadmium in the blood (percent of the administered dose) (A) and plasma (percent of Hg and/or Cd in blood) (B) of rats 1 h after the intravenous injection of a 0.5-µmol/kg dose of mercuric chloride and/or a 10-µmol/kg dose of cadmium chloride. All values are expressed as the mean ± SE for four to five animals per group. Asterisk indicates significantly different from the corresponding mean for the group of rats treated only with the 0.5-µmol/kg dose of mercuric chloride.

ministered dose of mercury was present in the total renal mass of the experimental group treated with both inorganic mercury and cadmium.

Renal Content of Cadmium In both groups of rats injected with the 10-µmol/kg dose of cadmium chloride, approximately 1.85% of the administered dose of cadmium was present in the total renal mass 24 h after treatment (Figure 5).

Concentration of Mercury in Renal Zones Renal cortical concentrations of mercury were on average approximately 16% lower in the rats

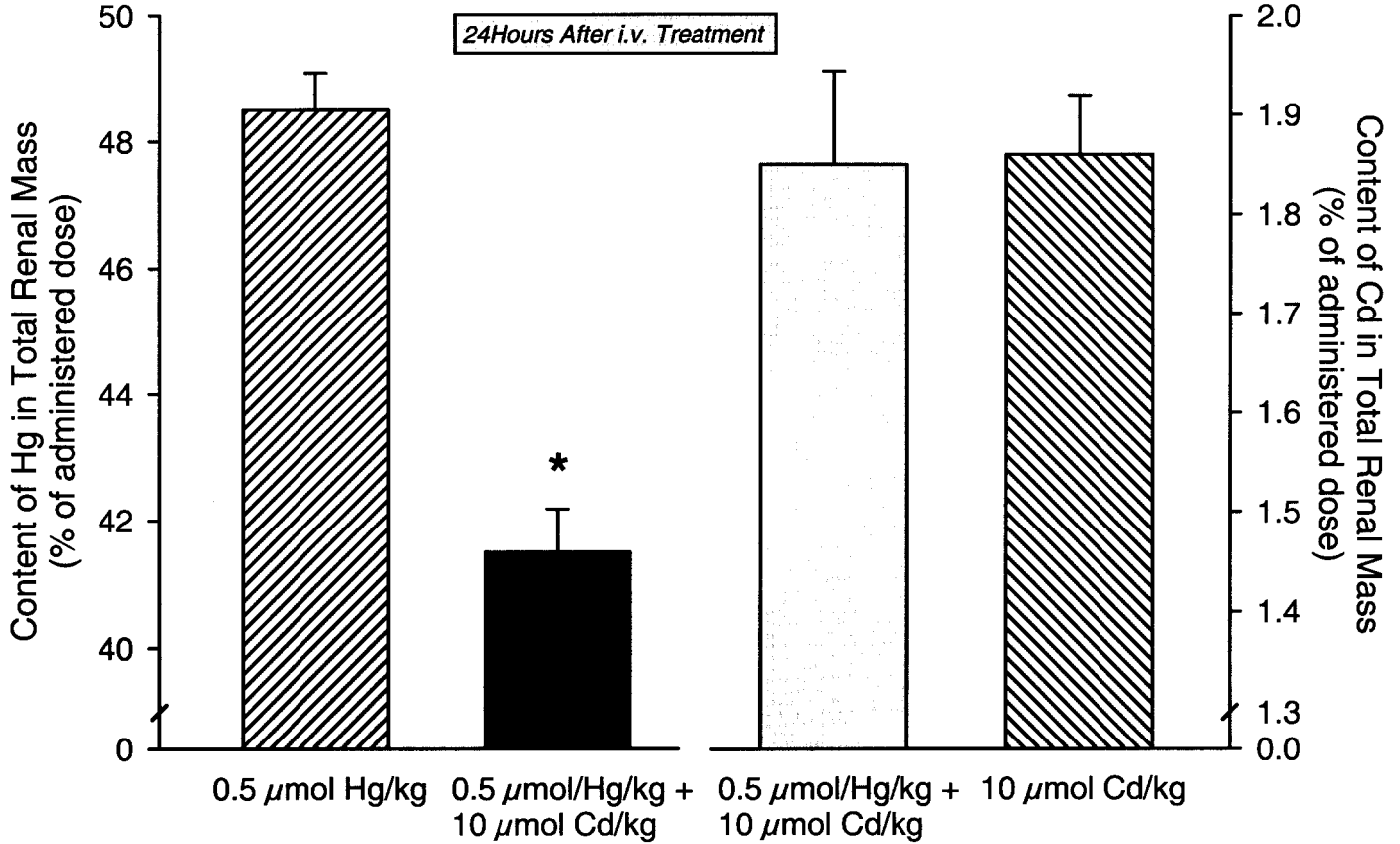

FIGURE 5. Content of inorganic mercury and/or cadmium (percent of the administered dose) in the total renal mass of rats 24 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride and/or a 10-μmol/kg dose of cadmium chloride. All values are expressed as the mean ± SE for four to five animals per group. Asterisk indicates significantly different from the corresponding mean for the group of rats treated only with the 0.5-μmol/kg dose of mercuric chloride.

treated with both inorganic mercury and cadmium than in the rats treated only with inorganic mercury (Figure 6A). However, no significant difference was detected in the concentration of mercury in the outer stripe of the outer medulla between these two groups of rats that received the 0.5-μmol/kg dose of inorganic mercury (Figure 6B).

Concentration of Cadmium in Renal Zones Mean concentrations of cadmium, in the renal cortex or the renal outer stripe of the outer medulla,

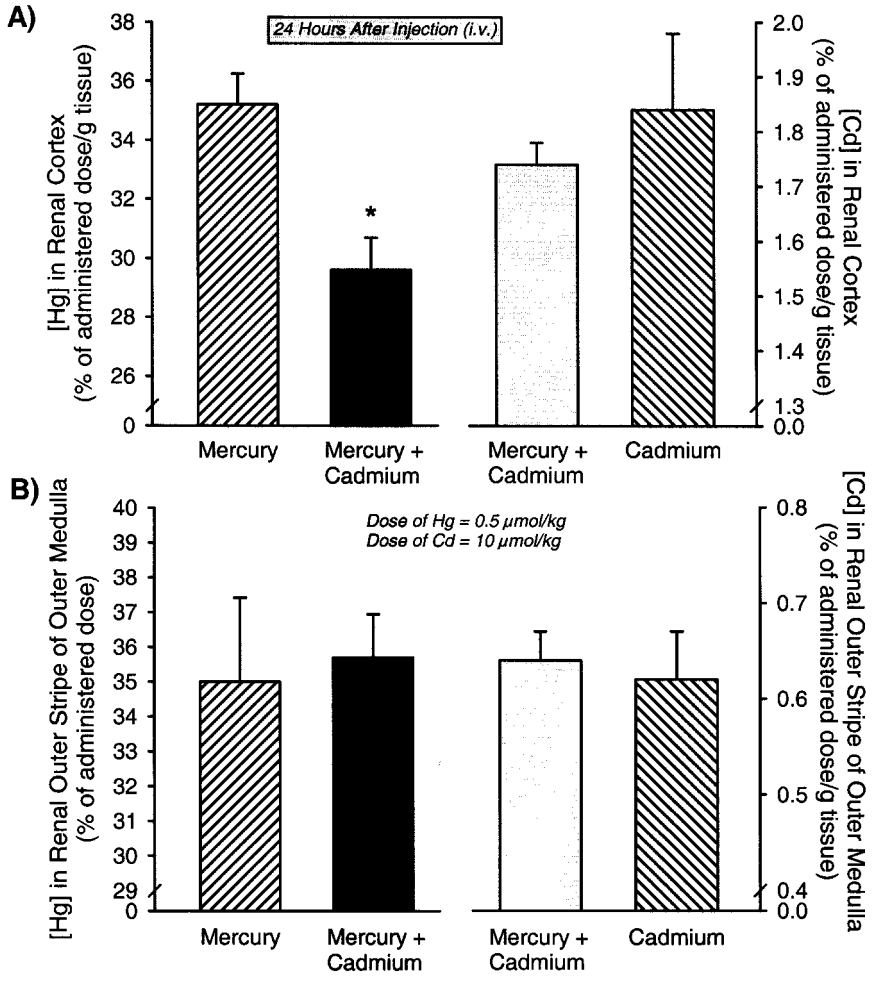

FIGURE 6. Concentration of inorganic mercury and/or cadmium (percent of the administered dose per gram tissue) in the renal cortex (A) and outer stripe of the outer medulla (B) of rats 24 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride and/or a 10-μmol/kg dose of cadmium chloride. All values are expressed as the mean ± SE for four to five animals per group. Asterisk indicates significantly different for the corresponding mean for the group of rats treated only with the 0.5-μmol/kg dose of mercuric chloride.

were similar in the two groups of rats injected with cadmium at 24 h after treatment (Figure 6, A and B).

Hepatic Content of Mercury In contrast to the findings obtained at 1 h after treatment, the hepatic content of mercury at 24 h in the groups injected with both inorganic mercury and cadmium was approximately 32% greater (rather than less) than that in the animals treated with only inorganic mercury (Figure 7).

Hepatic Content of Cadmium No significant difference in the hepatic content of cadmium was detected at 24 h after treatment between the 2 corresponding groups injected with cadmium chloride (Figure 7). However, the hepatic burden in both groups of rats was between 56 and 57% of the administered dose.

Content and Distribution of Mercury and Cadmium in Blood By the end of first 24 h after treatment, very little mercury or cadmium was present in the blood. In the animals that received 0.5 μmol inorganic mercury/kg, between 38 and 44% of the mercury in blood was present in the plasma (Figure 8). In contrast, only about 3.5% of the cadmium in blood was present in the plasma of the animals that received 10 μmol cadmium/kg. More importantly, there were no statistically significant differences in the content or distribution of inorganic mercury or cadmium between corresponding groups of rats.

Urinary and Fecal Excretion of Mercury Rats injected only with inorganic mercury excreted approximately 6% of the dose of mercury in the urine during the initial 24 h after treatment. In contrast, the rats treated with both inorganic mercury and cadmium excreted about 25% more mercury in the urine.

Although the rats treated with both inorganic mercury and cadmium excreted about 20% more mercury in the feces than the corresponding rats treated with just inorganic mercury, the difference in the fecal excretion of mercury between the two groups of animals was not statistically significant at $p < .05$.

Urinary and Fecal Excretion of Cadmium Very little cadmium was excreted in the urine in 24 h by the 2 groups of rats treated with cadmium. Only about 0.002 to 0.003% of the dose of cadmium was excreted in the urine by these animals.

Fecal excretion was the predominant means of excreting cadmium in both groups of rats treated with cadmium. In the rats treated only with cadmium, about 6.8% of the dose of cadmium was excreted in the feces in 24 h. In comparison, approximately 52% more cadmium was excreted in the feces by the group of rats treated with both inorganic mercury and cadmium.

DISCUSSION

In the present study, approximately 43% of the dose of inorganic mercury was present in the combined renal mass of rats 1 h after the intra-

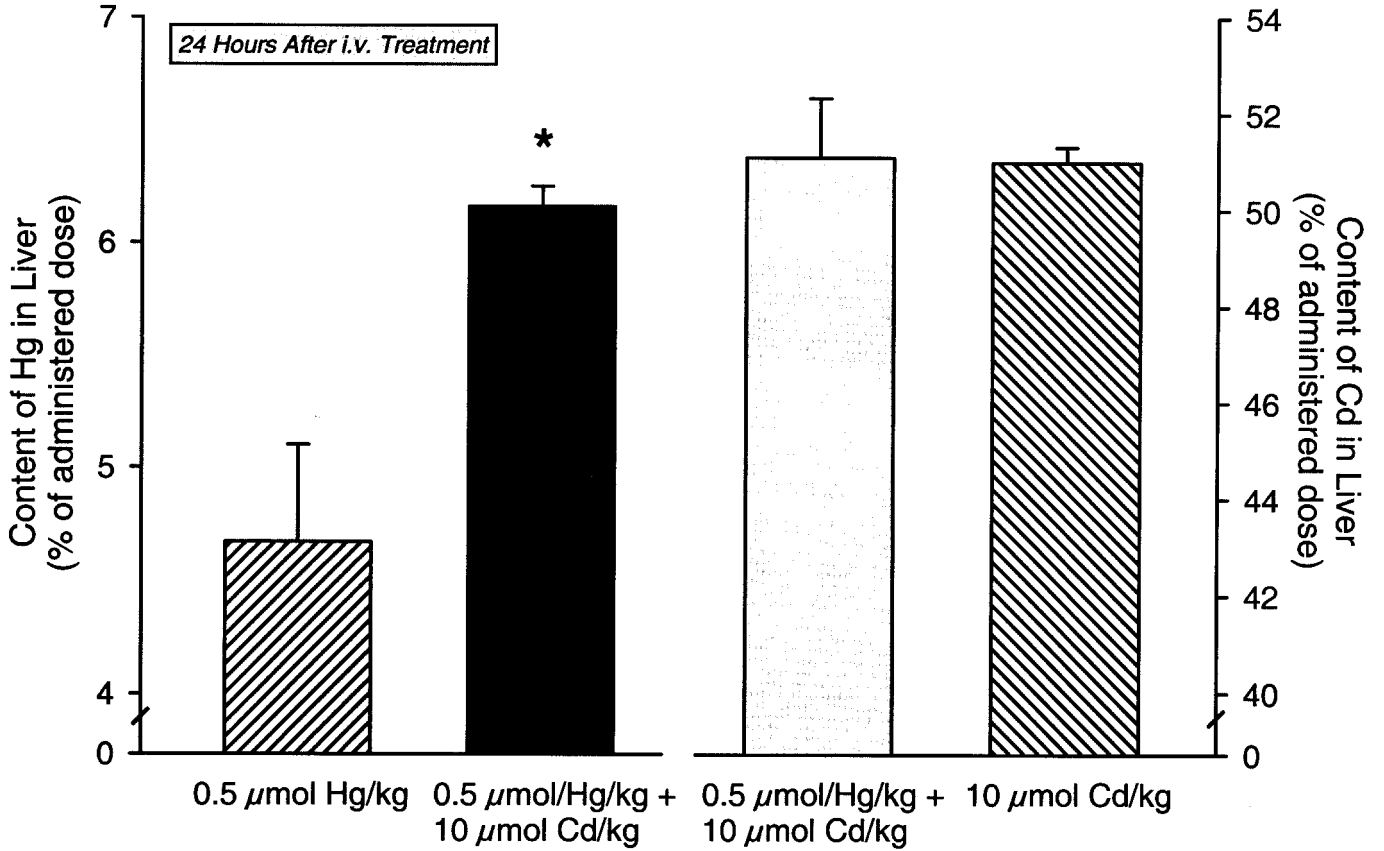

FIGURE 7. Content of inorganic mercury and/or cadmium (percent of the administered dose) in the liver of rats 24 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride and/or a 10-μmol/kg dose of cadmium chloride. All values are expressed as the mean ± SE for four to five animals per group. Asterisk indicates significantly different from the corresponding mean for the group of rats treated only with the 0.5-μmol/kg dose of mercuric chloride.

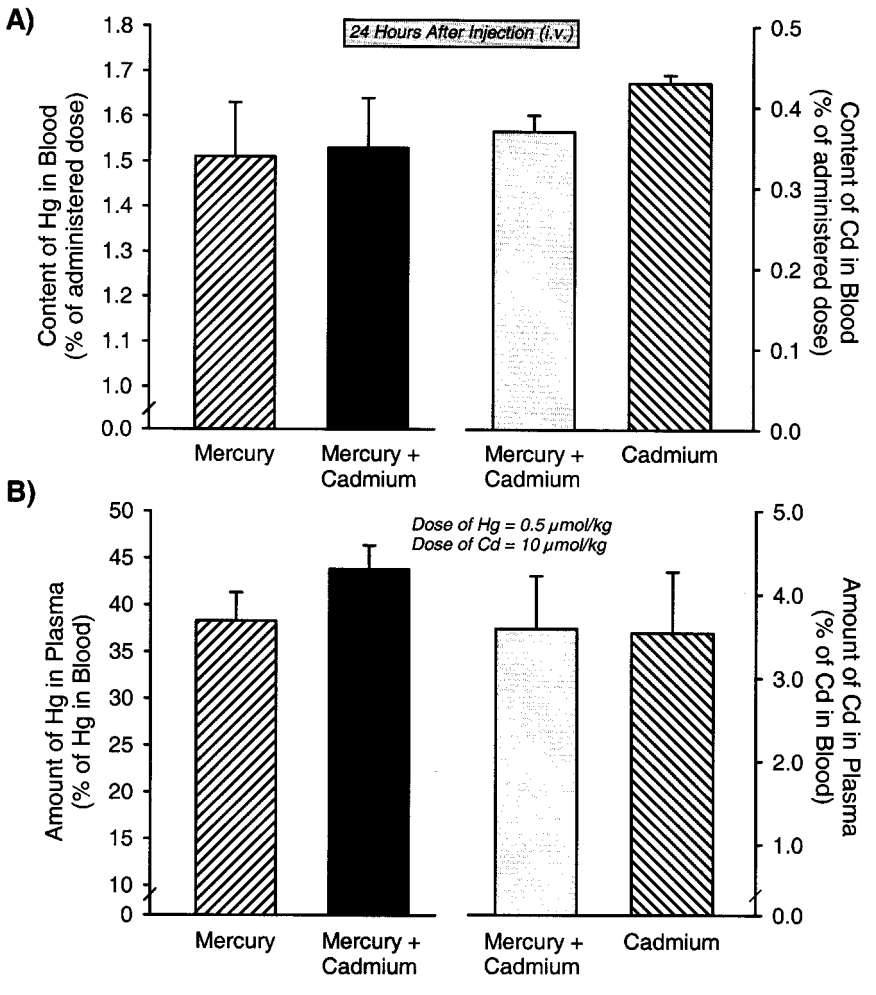

FIGURE 8. Content or amount of inorganic mercury and/or cadmium in the blood (percent of the administered dose) (A) and plasma (percent of Hg and/or Cd in blood) (B) of rats 24 h after the intravenous injection of a 0.5-µmol/kg dose of mercuric chloride and/or a 10-µmol/kg dose of cadmium chloride. All values are expressed as the mean ± SE for four to five animals per group.

venous administration of a 0.5-µmol/kg dose of mercuric chloride. The fraction of the dose detected in the kidneys (1 h after treatment) is consistent with that reported previously (Zalups & Barfuss, 1996, 1998a, 1998b). During the remainder of the initial 24 h after treatment with inorganic mercury, the renal burden of inorganic mercury increased to slightly more than 48% of the dose, confirming that the preponderance of the renal accumulation of inorganic mercury occurs during the initial hour subsequent to exposure (reviewed in Zalups & Lash, 1994; Zalups, 2000a). Moreover, the present findings confirm that the kidney is the primary target organ where

inorganic forms of mercury accumulate (reviewed in Zalups & Lash, 1994; Zalups 2000a).

When the 0.5-μmol/kg dose of inorganic mercury was coadministered with the 10-μmol/kg dose of cadmium, the net renal burden of inorganic mercury was reduced to only 33% of the dose 1 h after treatment, and 41% of the dose 24 h after treatment. According to the data obtained from the isolated renal zones, this reduction in the net renal accumulation of mercury was due primarily to decreased uptake and/or accumulation of mer-

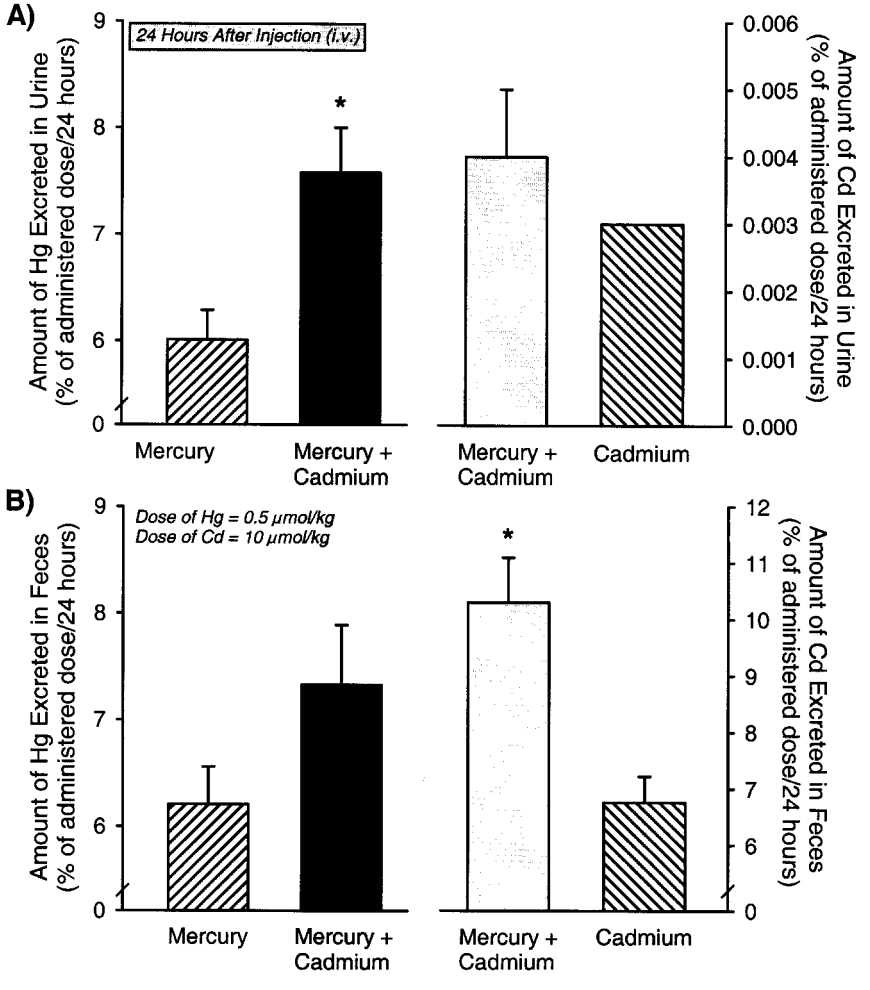

FIGURE 9. Amount of inorganic mercury and/or cadmium excreted (percent of the administered dose per 24 h) in the urine (A) and feces (B) by rats during the initial 24 h after the intravenous injection of a 0.5-μmol/kg dose of mercuric chloride and/or a 10-μmol/kg dose of cadmium chloride. All values are expressed as the mean ± SE for four to five animals per group. Asterisk indicates significantly different from the corresponding mean for the group of rats treated only with the 0.5-μmol/kg dose of mercuric chloride or 10-μmol/kg dose of cadmium.

cury in the renal cortex, although a significant reduction in the uptake and accumulation of mercury in the outer stripe of the outer medulla likely contributed to changes in the renal disposition of inorganic mercury during the first hour after coexposure. Overall, these findings indicate clearly that significant changes occur in the handling and accumulation of inorganic mercury in the kidneys, particularly in the renal cortex, during the initial 24 h after the coadministration of low doses of inorganic mercury and cadmium.

Although the precise mechanism(s) responsible for the reduction in the renal burden of inorganic mercury associated with coadministration of cadmium is/are not known at present, one possibility is that cadmium, in the extracellular (primarily intravascular) compartment, influences significantly the manner in which mercuric ions are taken up in the kidneys. The primary cellular targets where mercuric ions are taken up and accumulated in the kidneys are the epithelial cells lining the proximal tubule (Zalups, 1991a, 1991b). Recent evidence indicates that mercuric ions are transported into proximal tubular cells from the luminal compartment, via the actions of both sodium-dependent and sodium-independent amino transporters, through mechanisms involving molecular homology or "mimicry" (Cannon et al., 2000, 2001; Zalups & Barfuss, 1998a, 1998b; Zalups & Minor, 1995; Zalups et al., 1998). There are also several lines of evidence indicating that inorganic mercury is taken up at the basolateral membrane of proximal tubular cells by the probenecid and *p*-aminohippurate-sensitive organic anion transporter (OAT1) (Zalups, 1995; Zalups & Barfuss, 1995a; Zalups et al., 1998). Based on the fact that inorganic mercury is taken up at both luminal and basolateral membranes of proximal tubular epithelial cells, it is possible that cadmium may influence significantly the manner in which inorganic mercury is taken up into the apical and/or basal regions of proximal tubular cells.

Since cadmium ions (like mercuric ions) have a strong affinity for thiolate anions (Rabenstein, 1989), some of the cadmium in blood or the proximal tubular luminal fluid may be bound to ligands similar to or the same as those implicated in the renal tubular transport of inorganic mercury. Should this be the case, these conjugates of cadmium may serve as competitive substrates for, or allosteric inhibitors of, one or more of the transporters involved in the transport of mercuric ions into proximal tubular epithelial cells.

Two nonprotein thiols that have been implicated in the proximal tubular uptake of inorganic mercury are glutathione and cysteine (Zalups, 2000b), both of which are present in blood (in the reduced state) at low micromolar concentrations (Lash & Jones, 1985). We have demonstrated previously that when inorganic mercury is coadministered with, or conjugated to, either of these ligands at the time it is administered, the renal accumulation of mercury is enhanced significantly (Zalups & Barfuss, 1995b, 1995c). Similarly, Zalups (2000b) has also demonstrated recently that when cadmium is coadministered with cysteine or glutathione, the net renal uptake and accumulation of cadmium are enhanced significantly (Zalups, 2000b). These findings, and the fact that cadmium and mercury are both taken up predominantly by

proximal tubular cells in the kidneys, support the hypothesis that some of the same mechanisms involved in the renal tubular uptake of inorganic mercury may be involved in the renal tubular uptake of cadmium.

An alternate hypothesis for the altered renal disposition of mercury after coadministration with cadmium is that cadmium ions may enter into the renal epithelial cells in a more rapid and efficient (noncompetitive) manner than mercuric ions. This in turn would likely affect significantly the manner in which mercuric ions bind to various intracellular nucleophilic sites, resulting in a net decreased accumulation of inorganic mercury. It should be pointed out, however, that notwithstanding the current body of knowledge regarding the mechanisms by which mercuric ions gain access into proximal tubular cells, very little is known about the mechanisms by which cadmium ions gain access into these same target sites within the kidney. Consequently, it is virtually impossible, at present, to define the precise mechanisms by which cadmium influences the renal accumulation and handling of inorganic mercury.

If the decreased renal accumulation of inorganic mercury (associated with coadministration with cadmium) is in part due to a decrease in proximal tubular absorption of mercuric ions from the luminal compartment, one would predict there should be an associated increase in the urinary excretion of mercury, providing that there is no significant absorption of mercury at distal sites along the nephron. In fact, the net urinary excretion of mercury was indeed increased significantly (by approximately 24%) in the rats treated with both metals. Therefore, the urinary data support the hypothesis that coadministration of inorganic mercury and cadmium causes a decrease in the luminal absorption of inorganic mercury. However, the nature of the urinary data does not permit one to conclude with certainty that some of the increase in the urinary excretion of mercury was not, at least in part, due to some form of secretion of mercury into the luminal compartment. Additional studies will be needed to determine if coadministration of inorganic mercury and cadmium promotes secretion of mercuric ions along the proximal tubule.

Unlike the large fraction of the dose of inorganic mercury that accumulated in the kidneys of the rats treated with only inorganic mercury, no more than about 2% of the administered dose of cadmium was present in the total renal mass of the rats treated with only cadmium by the end of the initial 24 h after treatment. These data are consistent with previous dispositional findings obtained from rats treated with a similar dose of cadmium (Zalups, 2000b). What is particularly interesting, however, is the fact that coadministering cadmium with inorganic mercury did not result in any statistically significant changes in the renal disposition of cadmium.

As alluded to earlier, when the renal burden of each metal is expressed as a percentage of the administered dose, there are great differences between the renal burden of cadmium and the renal burden of inorganic mercury in the animals coadministered both metals. However, when the renal

burden of each metal is expressed in terms of the actual quantity of metals ions present in the kidneys, the renal burden of mercuric and cadmium ions is similar. More specifically, on average 41 nmol inorganic mercury and 38 nmol cadmium were present in the total renal mass of the rats studied 1 h after receiving both inorganic mercury and cadmium simultaneously. This similarity in the amount of mercuric and cadmium ions in the kidneys may be serendipitous, but on the other hand may have important mechanistic implications.

In addition to the reduced renal accumulation of mercury detected in the animals coadministered inorganic mercury and cadmium, significant changes were also detected in the hepatic accumulation and handling of inorganic mercury. During the initial hour after treatment, the net hepatic accumulation of mercury was diminished by approximately 33%. However, during the subsequent 23 h, the hepatic burden of mercury increased, which is in contrast to what occurs in the animals treated only with inorganic mercury. As a result, by the end of the initial 24 h after treatment, the hepatic burden of mercury in the animals coadministered both metals ended up being significantly greater than that in the corresponding rats treated with only inorganic mercury.

Normally, the hepatic accumulation of mercuric ions increases markedly in rats during the initial hours after exposure to a low dose of inorganic mercury (Zalups, 1993). Once a maximal burden of mercury has been reached in the liver (which can be as much as 8–10% of the dose), the amount of mercury in the liver decreases. Current data indicate that this decrease is likely due to export of mercuric conjugates of glutathione from within hepatocytes into biliary canaliculi (Ballatori & Clarkson, 1984). In the biliary canaliculi, the mercuric conjugates of glutathione are broken down enzymatically to mercuric conjugates of cysteine, which are delivered to the duodenum. There are also data indicating that inorganic mercury is secreted into the lumen of the small intestine (Zalups & Barfuss, 1996).

Recent data indicate that by the end of the initial 2 wk after exposure to a 0.5-μmol/kg dose of mercuric chloride, less than 2% of the dose is remaining in the liver, and that the hepatic retention (or elimination) of mercury correlates highly with the hepatic levels of metallothionein (MT)-1 and MT-2 protein or the relative rates of transcription of *MT-1* and *MT-2* genes. In contrast, as much as 40% of the dose of inorganic mercury is retained in the total renal mass by the end of the initial 2 wk following treatment (Zalups & Koropatnick, 2000). In addition, the data show that there is a very poor relationship between the renal levels of inorganic mercury and the renal levels of *MT*-1 and *MT*-2 or the rates of transcription of *MT-1* and *MT-2* genes in the kidneys.

Although there were no significant differences in the renal or hepatic burden of cadmium between the rats treated with both inorganic mercury and cadmium and those treated only with cadmium, the animals treated with both metals excreted about 51% more cadmium in the feces than the

animals treated with only cadmium. The reason for this altered fecal excretion of cadmium is not known.

In conclusion, simultaneous coexposure to low doses of inorganic mercury and cadmium causes significant changes in the renal and hepatic handling and disposition of mercury relative to that which occurs following exposure to just inorganic mercury alone. In contrast, the only obvious change in the disposition of cadmium following coexposure is a significant decrease in the fecal excretion of cadmium. The findings from the present study also tend to indicate that the toxicological profile of each metal may be altered significantly following coexposure to toxic levels of either, or both, metals. Additional studies are clearly warranted to better define the effects of different doses of each metal on not only the disposition but also the toxicity of the metals in the kidneys and liver, as well as other organs.

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