

DEPLETION OF GLUTATHIONE IN THE KIDNEY AND THE RENAL DISPOSITION OF ADMINISTERED INORGANIC MERCURY

RUDOLFS K. ZALUPS AND LAWRENCE H. LASH

Division of Basic Medical Sciences (R.K.Z.), Mercer University School of Medicine; and Department of Pharmacology (L.H.L.), Wayne State University School of Medicine

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ABSTRACT:

The primary aim of the present study was to evaluate the effects of different means of depleting glutathione (GSH) in the kidneys and liver on the renal and hepatic accumulation and disposition of a nontoxic dose of inorganic mercury. Renal and hepatic disposition of mercury were evaluated 1 hr after the intravenous administration of a 0.5 $\mu\text{mol/kg}$ dose of mercuric chloride in control rats and rats pretreated with acivicin, buthionine sulfoximine (BSO), or diethylmaleate (DEM). Pretreatment with acivicin or DEM caused significant decreases in the net renal accumulation of mercury during the first hour after the injection of mercuric chloride. The primary effects of these two pretreatments occurred in the renal cortex, although pretreatment with DEM also caused significant decreases in the concentration of mercury in the outer stripe of the outer medulla. Despite the fact that pretreatment with BSO caused a reduction in the renal content of GSH, comparable with that caused by DEM, pretreatment with BSO had no significant effect on the renal disposition of mercury. Pretreatment with acivicin, BSO, or DEM also caused significant decreases in measurable

reduced GSH, with BSO and DEM having the most pronounced effects. Injection of the nontoxic dose of mercuric chloride after pretreatment with acivicin resulted in slightly, but significantly, decreased hepatic content of mercury. Interestingly, pretreatment with BSO or DEM actually caused significant increases in the hepatic content of mercury 1 hr after the injection of mercuric chloride. We postulate that this effect was due to a diminished ability of hepatocytes to export mercuric conjugates of GSH out into either the bile or blood. The results of this study indicate that depletion of renal GSH by conjugation reactions between GSH and DEM leads to an acute reduction in the renal accumulation of inorganic mercury. However, the results also indicate that depletion of renal levels of GSH by inhibition of GSH synthesis does not affect acutely the ability of the kidneys to accumulate inorganic mercury. Thus, it seems that factors in addition to intracellular GSH status play an important role in the renal accumulation/retention of inorganic mercury.

It has been postulated by numerous investigators that modification, particularly depletion, of intracellular concentrations of GSH¹ in the kidneys has a significant influence on the renal disposition of inorganic mercury. Unfortunately, controversy arose because of differences in findings obtained from studies directed at testing this hypothesis. Depending on the mode by which renal GSH status had been modified and the dose of mercury administered, varied responses from decreased (1–3) to increased (4) renal accumulation of administered inorganic mercury have been reported in animals in which the renal concentration of GSH had been decreased.

In an attempt to shed some light on this controversy, the present study was designed to evaluate and compare, in rats, the effects of three different methods commonly used to deplete the renal concentrations of GSH on the renal disposition of inorganic mercury after the administration of a nontoxic dose of mercuric chloride. The primary aim of the present study was to test the hypothesis that mode of depletion of renal GSH is an important determinant in the renal disposition of inorganic mercury.

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¹ Abbreviations used are: GSH, glutathione; BSO, buthionine sulfoximine; DEM, diethylmaleate; GSSG, glutathione disulfide.

Send reprint requests to: Dr. Rudolfs K. Zalups, Division of Basic Medical Sciences, Mercer University School of Medicine, 1550 College Street, Macon, GA 31207.

A critical difference between this study and previous investigations on the effects of alterations in GSH status on the disposition of inorganic mercury in tissues and organs (particularly in the kidney) is that we used a nontoxic dose of inorganic mercury, whereas toxic doses were used in the previous studies (1–3). Hence, complications from the pathophysiological effects of toxic doses of inorganic mercury should not confound the results from the present study. Our result showed that, depending on the method by which intracellular concentrations of GSH are decreased (*i.e.* inhibition of GSH turnover with acivicin, inhibition of synthesis of GSH with BSO, or conjugation of GSH with DEM), there are differences in the renal and hepatic contents of inorganic mercury 1 hr after the administration of the nonnephrotoxic dose of mercuric chloride.

Materials and Methods

Animals and Groups. Forty male Sprague Dawley rats were used in the present study. They were purchased from Harlan Sprague-Dawley (Indianapolis, IN) at a weight of 175–200 g. After 3–4 days of acclimation, the animals were separated into groups of five.

There were four main types of groups used in the present study. One type 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. Two sets of each type of group were used. One set was used for determining renal and hepatic GSH status at the time that a nontoxic dose of mercuric chloride was administered. The other set was used to evaluate the effect of altering GSH status on the disposition of the injected mercury.

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

Pretreatments. As mentioned previously, 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 (5), which results in the inhibition of $\sim 97\%$ of the activity of γ -glutamyltransferase in the kidneys. First, the animals received a 10 mg/kg intraperitoneal 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 either received a nontoxic 0.5 $\mu\text{mol/kg}$ intravenous dose of mercuric chloride or were anesthetized with a 50 mg/kg dose of sodium pentobarbital (intraperitoneally) to obtain samples of kidneys and liver for determination of GSH status.

BSO and DEM were chosen to deplete GSH in the kidneys and liver because depletion could be attained by different mechanisms. Depletion of GSH after pretreatment with BSO is accomplished by inhibition of γ -glutamylcysteine synthetase (6), 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 described previously by Baggett and Berndt (1). 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. Two hours after pretreatment with either BSO or DEM, the animals either received the nontoxic 0.5 $\mu\text{mol/kg}$ intravenous dose of mercuric chloride or were anesthetized with a 50 mg/kg dose of sodium pentobarbital (intraperitoneally) to obtain samples of kidneys and liver for determination of GSH status. Twenty-four hours before experimentation, the BSO- and DEM-pretreated rats received a priming dose of DEM intraperitoneally. On examination, after the administration of acivicin, BSO, or DEM, the gross structural characteristics of the kidneys seemed normal, even 1 hr after the administration of the 0.5 $\mu\text{mol/kg}$ dose of mercuric chloride. This suggests that no toxic effects occurred as a result of the pretreatments used during the periods studied.

Evaluation of GSH Status in Samples of Kidneys and Liver. As stated previously, one set of control, acivicin-pretreated, BSO-pretreated, and DEM-pretreated rats were anesthetized with sodium pentobarbital immediately after their respective pretreatment. Once anesthesia had been induced, the kidneys and liver were removed, cleared of fat and connective tissue, and weighed quickly. Subsequently, a half kidney and a sample of renal cortex and outer stripe of the outer medulla were obtained from the left kidney and a 1-g sample of liver was obtained. Immediately after each sample of tissue was obtained from each animal, it was placed quickly into a cold (4°C) solution containing 1 mM bathophenanthroline disulfonic acid in 10% (v/v) perchloric acid. Bathophenanthroline was added to inhibit autooxidation during processing of the samples. The samples of one half kidney and liver were placed in 10 ml of the solution, whereas the other renal samples were placed in 1.5 ml of the solution. All of the samples were homogenized in a glass/Teflon homogenizer with a variable speed motor. Homogenates were centrifuged at 4°C at 1,250g for 10 min. The supernatants (perchloric acid extracts) were used for subsequent analyses.

Concentrations of GSH and cysteine were measured as *S*-carboxymethyl and *N*-dinitrophenyl derivatives, and GSSG was measured as the *N,N*-bis-dinitrophenyl derivative using HPLC. Separations were achieved with a μ Bondapak amine 10- μm cartridge (8 mm \times 10 cm) (Waters Associates, Milford, MA) with a Waters model 600E multisolvent delivery system using a methanol-acetate mobile phase and gradient elution. Derivatives were detected at 365 nm on a Waters model 490 detector and were quantified with respect to standards using a Waters model 745 data module. Concentrations of GSH, GSSG, and cysteine are expressed in mole units per gram of wet weight of tissue.

Injection of Inorganic Mercury. The other set of control, acivicin-pretreated, BSO-pretreated, and DEM-pretreated rats (not used in the assessment of GSH status) was administered a 0.5 $\mu\text{mol/kg}$ dose of mercuric chloride into the femoral vein after pretreatment. Radioactive inorganic mercury in the form of mercuric chloride ($^{203}\text{HgCl}_2$; Buffalo Materials Corp., Buffalo, NY) was added to the injection solution containing nonradioactive mercury. The injection

solution was designed to deliver 0.5 $\mu\text{mol Hg}^{2+}/\text{kg}$ and 4 $\mu\text{Ci }^{203}\text{Hg}^{2+}/\text{kg}$ in 2.0 ml normal saline (0.2-ml injection volume/100 g body weight).

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 50 mg/kg dose of sodium pentobarbital (intraperitoneally). 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 preweighed 12 \times 75 mm, round-bottom, gamma-counting tube. The other 1.0 ml of whole blood was centrifuged at 10,000g 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 and liver 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 outer and inner stripes of the inner medulla were obtained. A 1-g sample of liver was also obtained.

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 $\sim 50\%$ for $^{203}\text{Hg}^{2+}$. The content of mercury in the samples was calculated by dividing the activity of $^{203}\text{Hg}^{2+}$ (dpm) in the sample by the specific activity of $^{203}\text{Hg}^{2+}$ (dpm/nmol) in the injection solution. Concentrations of mercury in the tissues are expressed as percentage of the administered dose per gram of tissue, and the content of mercury in organs is expressed simply as a percentage of the administered dose. The total volume of blood in rats was estimated to be $\sim 6\%$ of body weight.

The 1-hr time point after the administration of mercuric chloride was chosen for measurements of the disposition of inorganic mercury, because the kinetics for the uptake of mercury in the kidney and liver are greatest during this period. During this initial hour after the injection of a nontoxic dose of mercuric chloride, $\sim 80\text{--}85\%$ of the total renal burden of mercury that is present 24 hr after mercuric chloride is taken up by the kidney. Moreover, the 1-hr time point was chosen, because 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.

Statistical Analyses. Statistical differences between means, of any parameter measured, for the four groups of rats studied were assessed using a one-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 percentage score. The level of significance for any of the statistical procedures used was chosen *a priori* to be $p < 0.05$.

Results

Concentration of GSH in Renal Tissues. Weights of the animals in which GSH status was evaluated, and the weights of their kidneys and liver are presented in table 1.

Decreased concentrations of GSH in the left kidney were detected in all rats that were pretreated with acivicin, BSO, or DEM (fig. 1A). However, the greatest decreases occurred in the rats that were pretreated with BSO or DEM. In these animals, the concentration of GSH in the left kidney was $\sim 54\text{--}66\%$ lower than that in the control rats. No significant difference in the renal concentration of mercury was detected between the rats pretreated with BSO and the rats pretreated with DEM. All differences mentioned in this section are statistically significant unless stated otherwise.

Just as was the case at the level of the whole kidney, pretreatment with acivicin, BSO, or DEM caused significant decreases in the concentration of GSH in the renal cortex. However, the effect of acivicin was slightly more prominent in the renal cortex than at the

TABLE 1
Concentration of GSSG and cysteine (CYS) in the kidneys and liver of rats treated with compounds that alter renal and hepatic GSH status

Group	Animal Body Weight	Weight of Left Kidney	Weight of Right Kidney	Weight of Liver	[GSSG] in Kidney	[GSSG] in Renal Cortex	[GSSG] in Renal OS/OM	[GSSG] in Liver	[CYS] in Kidney	[CYS] in Renal Cortex	[CYS] in Renal OS/OM	[CYS] in Liver
	g	g	g	g	$\mu\text{mol/g}$	$\mu\text{mol/g}$	$\mu\text{mol/g}$	$\mu\text{mol/g}$	$\mu\text{mol/g}$	$\mu\text{mol/g}$	$\mu\text{mol/g}$	$\mu\text{mol/g}$
Control N = 5	219 ± 11	0.93 ± 0.06	0.93 ± 0.07	9.56 ± 0.64	0.032 ± 0.002	0.047 ± 0.005	0.018 ± 0.007	0.106 ± 0.130	0.21 ± 0.02	0.33 ± 0.05	0.39 ± 0.03	0.11 ± 0.01
Acivicin N = 5	235 ± 19	0.90 ± 0.06	0.90 ± 0.06	9.61 ± 0.50	0.017 ± 0.002*	0.032 ± 0.004	0.017 ± 0.003	0.030 ± 0.003*	0.13 ± 0.02*	0.21 ± 0.05*	0.22 ± 0.02*	0.05 ± 0.01
BSO N = 5	204 ± 11	0.81 ± 0.05	0.81 ± 0.05	6.84 ± 0.52	0.008 ± 0.001**	0.008 ± 0.003**	0.018 ± 0.004	0.018 ± 0.004*	0.12 ± 0.01**	0.24 ± 0.03	0.15 ± 0.01*	0.08 ± 0.02
DEM N = 5	199 ± 8	0.82 ± 0.04	0.82 ± 0.05	6.49 ± 0.47	0.078 ± 0.014***	0.036 ± 0.013	0.054 ± 0.012***	0.025 ± 0.001*	0.19 ± 0.02***	0.13 ± 0.01	0.22 ± 0.03*	0.09 ± 0.02

Values represent mean ± SE for five rats. OS/OM, outer stripe of outer medulla. Consult with *Materials and Methods* for details of injections and treatments. * Significantly different ($p < 0.05$) from the corresponding mean for the control group. ** Significantly different ($p < 0.05$) from the corresponding means for the control group and the group treated with acivicin. *** Significantly different ($p < 0.05$) from the corresponding means for the control group, the group treated with acivicin, and the group treated with both BSO. **** Significantly different ($p < 0.05$) from the corresponding means for the group treated with acivicin and the group treated with BSO.

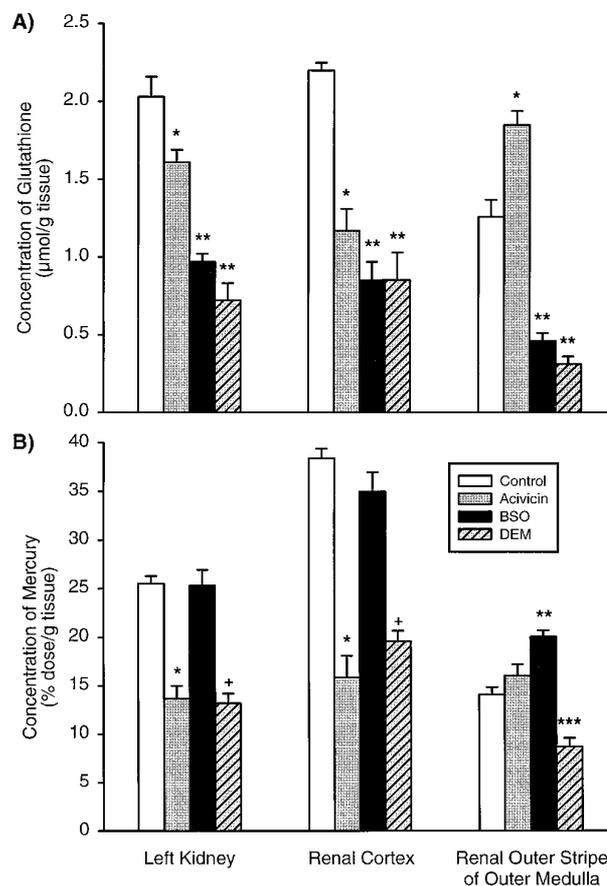


FIG. 1. Concentration of GSH in renal tissues (A) from groups of control rats and rats pretreated with acivicin, BSO, or DEM, and concentration of mercury in renal tissue (B) from groups of control rats and rats pretreated with acivicin, BSO, or DEM 1 hr after the intravenous injection of a 0.5 $\mu\text{mol/kg}$ dose of mercuric chloride.

See *Materials and Methods* for details of pretreatments and handling of tissues. Values are mean ± SE. * Significantly different ($p < 0.05$) from the corresponding mean for the control group. ** Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with acivicin. *** Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats, rats pretreated with acivicin, and rats pretreated with BSO.

level of the whole kidney. In the rats pretreated with acivicin, the mean renal concentration of GSH was ~45% lower than that in control rats. In addition, the mean concentration of GSH in the renal cortex of the rats pretreated with either BSO or DEM was ~64% lower than that in the control rats.

A slightly different pattern of effect was detected in the samples of the outer stripe of the outer medulla. Unlike in the other renal zones, pretreatment with acivicin actually caused an increase in the concentration of GSH. The mean concentration of GSH in the rats pretreated with acivicin was ~38% greater than that in the control rats. In the rats pretreated with either BSO or DEM, the mean concentration of GSH in the renal outer stripe of the outer medulla was between 65–77% lower than that in control rats.

Concentrations of Mercury in Renal Tissues. Body weights and the weights of the kidneys and liver in the animals that received the 0.5 $\mu\text{mol/kg}$ dose of mercuric chloride are presented in table 2.

Pretreatment with either acivicin or DEM, but not with BSO, resulted in decreased concentrations of mercury at the level of the whole kidney 1 hr after the intravenous administration of a nontoxic

TABLE 2

Concentration of injected mercury in renal zones and blood of rats treated with compounds that alter renal and hepatic GSH status

Group	Animal Body Weight	Weight of Left Kidney	Weight of Right Kidney	Weight of Liver	[Hg ²⁺] in IS/OM	[Hg ²⁺] in Inner Medulla	[Hg ²⁺] in Blood
	g	g	g	g	% dose/g	% dose/g	% dose/g
Control							
N = 5	208 ± 4	0.77 ± 0.03	0.79 ± 0.04	9.53 ± 0.25	1.00 ± 0.11	1.25 ± 0.10	1.06 ± 0.04
Acivicin							
N = 5	205 ± 2	0.78 ± 0.01	0.80 ± 0.02	9.13 ± 0.31	1.36 ± 0.08	1.80 ± 0.10	1.39 ± 0.09
BSO							
N = 5	195 ± 3	0.81 ± 0.03	0.80 ± 0.03	7.89 ± 0.45	1.33 ± 0.14	1.82 ± 0.47	1.04 ± 0.07
DEM							
N = 5	188 ± 2	0.74 ± 0.02	0.75 ± 0.01	6.70 ± 0.17	1.18 ± 0.07	1.75 ± 0.19	1.41 ± 0.04

Values represent mean ± SE for five rats. IS/OM, inner stripe of outer medulla. Consult with *Materials and Methods* for details of injections and treatments. All animals received an intravenous 0.5 μmol/kg dose of mercuric chloride containing 4 μCi/kg ²⁰³Hg²⁺ after being pretreated with 1 of the 4 protocols used. The disposition of inorganic mercury in the renal tissues and blood was evaluated 1 hr after injection of mercuric chloride.

0.5 μmol/kg dose of mercuric chloride (fig. 1B). Pretreatment with either acivicin or DEM caused the renal concentration of mercury to be ~46–48% less than that in the control rats or the rats pretreated with BSO.

A pattern of accumulation of mercury, similar to that found at the level of the of whole kidney, was detected in the samples of renal cortex. Pretreatment with acivicin or DEM, but not BSO, resulted in decreased concentrations of mercury in the renal cortex. The renal cortical concentrations of mercury in the rats pretreated with acivicin or DEM were ~49–59% lower than those in the control rats.

Interestingly, pretreatment with BSO caused an approximate 43% increase, pretreatment with DEM caused an approximate 38% decrease, and pretreatment with acivicin had no effect on the concentration of mercury in the outer stripe of the outer medulla relative to that in control rats.

The effects of the three pretreatments on the disposition of mercury in the inner stripe of the outer medulla and inner medulla are presented in table 2. Very little mercury generally accumulates in these two zones, which is confirmed by the present data. There did seem to be somewhat greater concentrations of mercury in both of these renal zones after any of the pretreatments. However, the differences between the means are not statistically significant at $p < 0.05$.

Concentration of GSH and Mercury in the Liver. Significant effects on hepatic concentrations of GSH were detected in rats pretreated with acivicin, BSO, or DEM (fig. 2A). All three forms of pretreatment caused decreases in the hepatic concentration of GSH. However, the greatest effects were detected in the rats pretreated with BSO or DEM. The mean hepatic concentration of GSH was ~61% lower in the rats pretreated with BSO and ~91% lower in the rats pretreated with DEM than in the control rats.

Mixed effects of the three forms of pretreatment on the hepatic concentration of mercury were detected 1 hr after the intravenous injection of the 0.5 μmol/kg dose of mercuric chloride (fig. 2B). In the rats pretreated with acivicin, the hepatic concentration of mercury was ~19% less than that in the control rats. By contrast, the hepatic concentration of mercury increased in the rats pretreated with either BSO or DEM. In fact, the concentration of mercury was ~51% greater in the rats pretreated with BSO and ~76% greater in the rats pretreated with DEM than in the control rats.

Concentrations of GSSG in Renal and Hepatic Tissues. Concentrations of GSSG in the kidney decreased (relative to that in control rats) in the rats pretreated with acivicin or BSO and increased in the rats pretreated with DEM (table 1). Only in the rats pretreated with BSO was there a significant effect on the concentration of GSSG

in the renal cortex. Pretreatment with BSO caused the concentration of GSSG to decrease in this zone. By contrast, only in rats pretreated with DEM was there a significant effect on the concentration of GSSG in the renal outer stripe of the outer medulla. Pretreatment with DEM caused the concentration of GSSG to increase in this zone.

In rats pretreated with BSO or DEM, significant changes in the concentration of GSSG in the liver were detected (table 1). These pretreatments caused the mean hepatic concentration of GSSG to decrease.

Concentrations of Cysteine in Renal and Hepatic Tissues. Rats pretreated with acivicin or BSO had lower concentrations of cysteine in their kidneys than control rats (table 1). Decreased concentrations of cysteine, relative to control values, were also detected in the renal cortex of the rats pretreated with acivicin or DEM and in the renal outer stripe of the outer medulla of the rats that were administered any of the three pretreatments.

Although the concentrations of cysteine tended to be lower in rats pretreated with acivicin, BSO, or DEM, the differences in the means for the hepatic concentration of cysteine between the four groups of animals were found not to be statistically significant (table 1).

Total Content of GSH in the Total Renal Mass and Liver. The content of GSH in the total renal mass of the rats pretreated with acivicin was ~25% lower than that in corresponding control rats (fig. 3A). Rats pretreated with acivicin also had ~31% less GSH in their liver relative to that in control rats (fig. 3C). In the rats pretreated with either BSO or DEM, the content of GSH in the total renal mass was ~60–68% lower than that in corresponding control rats and ~46–58% lower than in the corresponding rats treated with acivicin. In addition, the content of GSH in the liver of the rats pretreated with BSO was ~75% and 64% lower than that in control rats and the rats pretreated with acivicin, respectively. Moreover, the content of GSH in the liver of the rats treated with DEM was ~91%, 86%, and 63% lower than that in control rats, the rats pretreated with acivicin, and the rats pretreated with BSO, respectively.

Total Content of Mercury in the Total Renal Mass and Liver. Significant effects on the content of mercury in the total renal mass 1 hr after the injection of the 0.5 μmol/kg dose of mercuric chloride were detected only in rats pretreated with acivicin or DEM (fig. 3B). The content of mercury in the total renal mass was ~46% lower in the rats pretreated with acivicin and ~51% lower in the rats pretreated with DEM than in the control rats.

Pretreatment with acivicin caused the total content of mercury in the liver to decrease, whereas pretreatment with either BSO or DEM caused the content of mercury in the liver to increase (fig. 3D). The

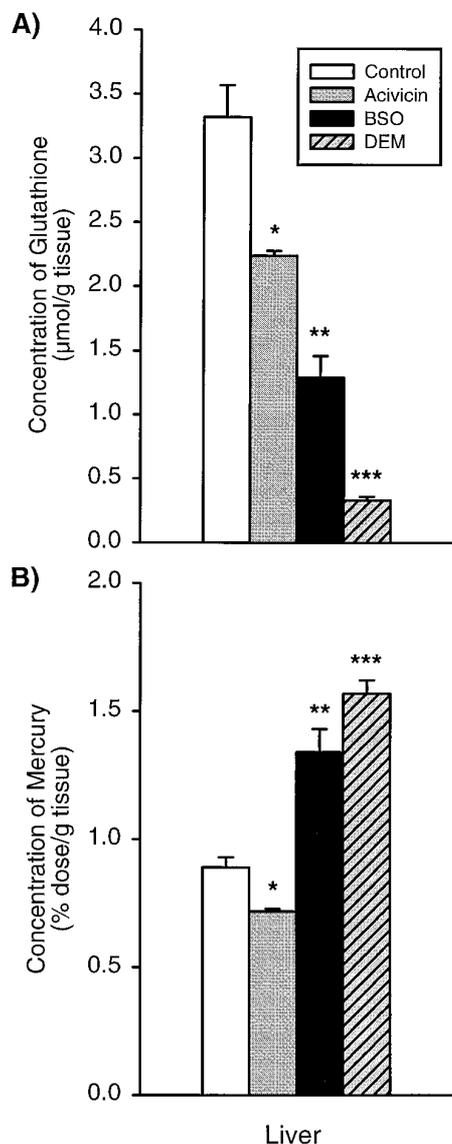


FIG. 2. Concentration of GSH in the liver (A) of groups of control rats and rats pretreated with acivicin, BSO, or DEM, and concentration of mercury in the liver (B) of groups of control rats and rats pretreated with acivicin, BSO, or DEM 1 hr after the intravenous injection of a 0.5 µmol/kg dose of mercuric chloride.

See *Materials and Methods* for details of pretreatments and handling of tissues. Values are mean \pm SE. * Significantly different ($p < 0.05$) from the corresponding mean for the control group. ** Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with acivicin. *** Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats, rats pretreated with acivicin, and rats pretreated with BSO.

mean hepatic content of mercury in the rats pretreated with acivicin was 22% lower than that in the control rats. By contrast, the mean hepatic content of mercury in the rats pretreated with either BSO or DEM was ~24% greater than that in the control rats.

Disposition of Mercury in the Blood. The influence of each pretreatment used on the concentration of mercury in the blood is presented in table 2. No statistically significant differences among the means for the concentration of mercury in blood were detected.

More mercury was present in the blood of the rats pretreated with acivicin or DEM, but not BSO, than was in the blood of the control

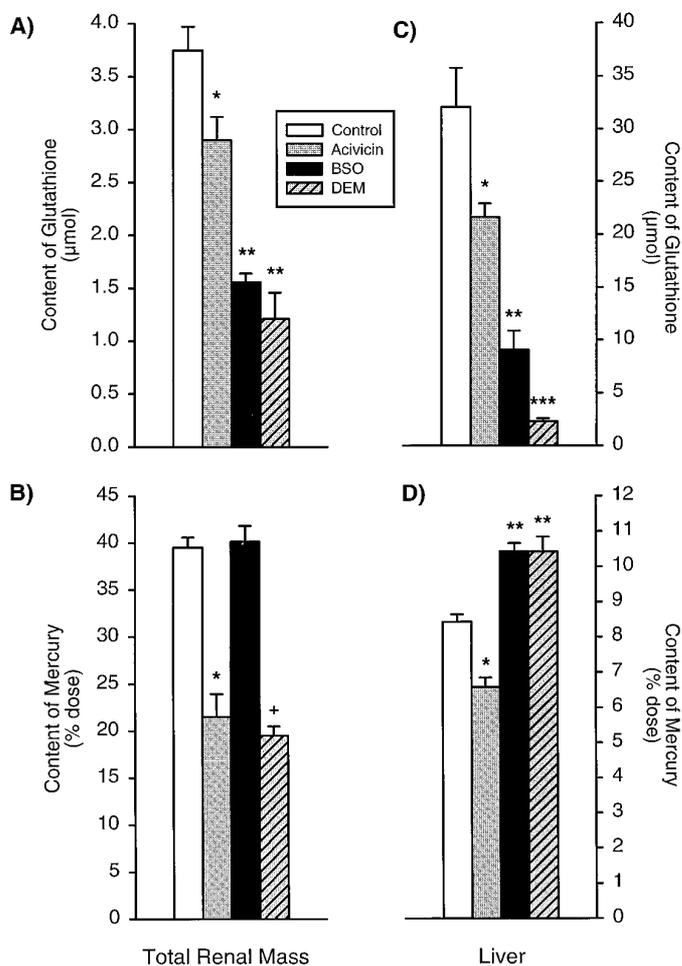


FIG. 3. Content of GSH in the total renal mass (A) and liver (C) of groups of control rats and rats pretreated with acivicin, BSO, or DEM, and the content of mercury in the total renal mass (B) and liver (D) of groups of control rats and rats pretreated with acivicin, BSO, or DEM 1 hr after the intravenous injection of a 0.5 µmol/kg dose of mercuric chloride.

See *Materials and Methods* for details of pretreatments and handling of tissues. Values are mean \pm SE. * Significantly different ($p < 0.05$) from the corresponding mean for the control group. ** Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with acivicin. *** Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats, rats pretreated with acivicin, and rats pretreated with BSO. + Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO.

rats (fig. 4). In addition, of the mercury in the blood, more was present in the plasma in the rats pretreated with DEM (but not acivicin or BSO) than in the control rats.

Discussion

In the present study, pretreatment of rats with acivicin, BSO, and DEM caused significant changes in renal GSH status, particularly in the cortex and outer stripe of the outer medulla. All three pretreatments led to a precipitous reduction in the overall renal concentration of GSH. Pretreatment with BSO and DEM was most effective in causing reductions in the overall renal concentration of GSH.

Interestingly, pretreatment with acivicin caused a significant depletion of GSH in the renal cortex, but led to a significant increase in the concentration of GSH in the renal outer stripe of the outer medulla. The fall in the concentration of GSH in the cortex would seem to be

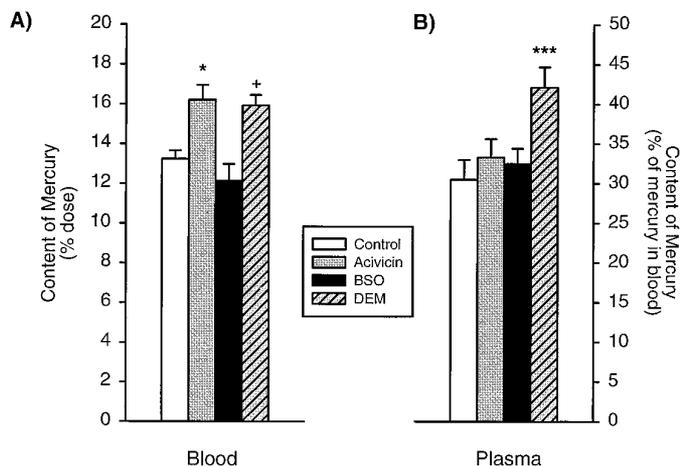


FIG. 4. Content of mercury in the blood (A) and the amount of mercury in blood present in the plasma (B) of groups of control rats and rats pretreated with acivicin, BSO, or DEM 1 hr after the intravenous injection of a 0.5 $\mu\text{mol/kg}$ dose of mercuric chloride.

See *Materials and Methods* for details of pretreatments and handling of tissues. Values are mean \pm SE. * Significantly different ($p < 0.05$) from the corresponding mean for the control group. *** Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats, rats pretreated with acivicin, and rats pretreated with BSO. + Significantly different ($p < 0.05$) from the means for the corresponding groups of control rats and rats pretreated with BSO.

consistent with the actions of acivicin. Because the compound significantly inhibits the actions of the γ -glutamyltransferase lining the brush-border membrane of proximal tubules (5), a significant fraction of filtered and secreted GSH in the proximal tubular lumen is not enzymatically broken down to cysteine, which is normally reabsorbed very rapidly and efficiently by the sodium-dependent neutral amino acid transport system. By reducing the pool of reabsorbable cysteine, which serves as the rate-limiting substrate involved in the biosynthesis of GSH, intracellular depletion of GSH occurs. Consistent with this notion are the findings from the present study indicating that the concentrations of cysteine in the cortex were reduced significantly after pretreatment with acivicin. The concentration of cysteine in the renal outer stripe of the outer medulla was also decreased significantly after pretreatment with acivicin, and yet the concentration of GSH in this zone of the kidney increased. It is not exactly clear as to what is responsible for this heterogeneous response. However, one possibility is that as the intraluminal concentration of GSH becomes sufficiently high enough in the terminal segments of the proximal tubule, as a result of the diminished enzymatic degradation of the tripeptide, an unmasking of a reabsorptive mechanism involving the uptake of GSH as an intact tripeptide occurs. Although it is unlikely that any luminal reabsorption of GSH, as an intact tripeptide, occurs *in vivo* under normal homeostatic conditions, uptake of GSH in terminal segments of the proximal tubule during near complete inhibition of the γ -glutamyltransferase cannot be ruled out at present. Studies on isolated proximal tubular cells from the rat (7, 8) have characterized sodium-dependent uptake and cellular accumulation of intact GSH. However, this uptake seems to occur mainly at the basolateral membrane (9). It should also be mentioned that there is evidence for dipeptide transport across the luminal membrane. In a recent study by Barfuss *et al.* (10), it was shown that the dipeptide glycylsarcosine, which is resistant to dipeptidase activity, can be transported across the luminal membrane of proximal tubular epithelial cells in an intact form.

When a nontoxic dose of mercuric chloride had been administered

after pretreatment with acivicin, an approximate 50% reduction in accumulation of mercury resulted during the first hour after injection of mercury. The decreased renal accumulation of mercury was due almost exclusively to diminished uptake and/or accumulation of mercury in the renal cortex, inasmuch as the concentration of mercury in the outer stripe of the outer medulla, as well as the other zones, was not affected significantly by pretreatment with acivicin. Diminished uptake of mercury after pretreatment with acivicin is also consistent with findings from other studies (2, 11–14). These findings indicate that pretreatment with acivicin causes the renal uptake of mercury to decrease significantly, and causes the urinary excretion of mercury and GSH to increase in rats and mice. In addition, the findings from these studies provide substantial evidence that the primary luminal mechanism involved in the uptake of mercury along proximal tubular epithelial cells is dependent on the activity of the γ -glutamyltransferase on the brush-border membrane.

Significant depletion of renal levels of GSH (by ~60–68%) by pretreatment with DEM, but not BSO, also caused a near 50% reduction in the renal content of mercury by the end of the first hour after the injection of the 0.5 $\mu\text{mol/kg}$ dose of mercuric chloride. This depletion was due to decreased uptake and/or accumulation of mercury in both the cortex and outer stripe of the outer medulla. Pretreatment with BSO had no effect on concentration of mercury at the level of the whole kidney or in the renal cortex, despite the fact the level of depletion of renal GSH was similar to that attained with the use of DEM. It did, however, cause a slight, but significant increase in the concentration of mercury in the outer stripe of the outer medulla.

The findings from the present study have rather significant implications with respect to the effects of depletion of renal GSH and the renal disposition of administered inorganic mercury. First of all, they indicate that, when renal levels of GSH are depleted by a mechanism involving conjugation, there is a decrease in the net accumulation of mercury shortly after exposure to nontoxic levels of inorganic mercury. In reality, the term depletion should be used loosely under these circumstances, inasmuch as the total intracellular number of molecules of GSH is probably not affected significantly, but rather, the free sulfhydryl group on the cysteinyl residue of a significant number of molecules of GSH is oxidized in a conjugation reaction with DEM. On the other hand, the present findings indicate that when intracellular concentrations of GSH are depleted in the renal cortex and outer stripe of the outer medulla by inhibiting the synthesis of new GSH with BSO, the net renal accumulation of mercury is not affected significantly during the first hour after exposure to nontoxic levels of inorganic mercury. It is unclear at present as to why the apparent net renal cortical accumulation of mercury remains unchanged during the inhibition of synthesis of new GSH. One partial explanation is that cysteinyl residues arising from the intraluminal degradation of filtered GSH, or preexisting GSH that was secreted into the lumen, help maintain temporarily free thiol status in the proximal tubular epithelial cells after they are reabsorbed. This hypothesis is supported to a limited degree by the data on the concentration of cysteine in the renal cortex, which indicate that there was not a statistically significant change in the concentration of cysteine in the renal cortex after pretreatment with BSO. The use of a nonnephrotoxic dose of mercuric chloride in the present study thus allowed us to examine effects of altered thiol status on the renal disposition of inorganic mercury without the complications of any apparent diminution of renal function.

In addition to low molecular weight thiols, such as GSH and cysteine, protein-sulfhydryls located on the plasma membrane and the intracellular compartment are also important ligands for inorganic

mercury. In fact, protein-sulfhydryl groups likely account for a significant portion of the total intracellular ligands for mercuric ions. Because protein-sulfhydryls are present at substantially higher amounts than low molecular weight thiols, the changes in the intracellular concentrations of GSH and cysteine that were produced by the pretreatments are unlikely to cause changes in their amounts during the 1-hr time frame for these experiments. Hence, variations in the renal and hepatic contents of inorganic mercury caused by the pretreatments should be due to changes in the concentrations of low molecular weight thiols and not to compensatory changes in the amount of protein-sulfhydryls.

Effects of depletion of renal GSH by DEM and BSO on the renal disposition of administered mercury have been studied by several groups of investigators. However, varied results have been reported due to one factor or another. For example, Johnson (3) demonstrated that depletion of nonprotein sulfhydryls resulted in diminished renal uptake of inorganic mercury after the administration of a highly nephrotoxic 15 mg/kg dose of mercuric chloride. Baggett and Berndt (1) also reported that depletion of renal levels of nonprotein-sulfhydryls by pretreatment with DEM alone or in combination with BSO caused decreases in the renal accumulation of inorganic mercury during the initial hours after the administration of a nephrotoxic 4.0 mg/kg dose of mercuric chloride. Depletion of nonprotein-sulfhydryl groups in the kidney by pretreatment with DEM has also been reported to cause a reduction in the renal accumulation of mercury during the initial 2 hr after the administration of methyl mercury (15). The previously mentioned findings are consistent with those from the present study. By contrast, Girardi and Elias (4) reported in a more recent study increased net renal accumulation of mercury in rats given a nephrotoxic 5.0 mg/kg dose of mercuric chloride when renal levels of GSH were depleted by ~25% after pretreatment with DEM. In another recent study, depletion of renal GSH by pretreatment with BSO caused the net renal accumulation of mercury to decrease in mice after exposure to mercury vapor (16). One of the confounding factors that significantly affects the ability to interpret the data from some of these previous studies is that nephrotoxic doses of inorganic mercury were used. When toxic doses of mercury are used, it becomes extremely difficult to dissect out the influence of the toxic effects of mercury from the effects of the actual depletion of GSH on the renal disposition of both mercury and GSH. It should be emphasized that the present study is apparently the first to examine the effects of acivicin, DEM, or BSO on the renal disposition of inorganic mercury in rats injected with a nontoxic dose of mercuric chloride.

In addition to having effects on renal GSH status, all three pretreatments used in the present study caused the concentration and content of GSH in the liver to decrease. By far, pretreatment with DEM and then BSO were most effective in depleting hepatic levels of GSH. Pretreatment with acivicin caused a slight but significant decrease in the net accumulation of mercury. However, pretreatment with either BSO or DEM increased substantially the net hepatic accumulation of mercury. The mechanism by which acivicin causes decreased hepatic accumulation of mercury may be similar to the one responsible for decreased renal uptake and accumulation of mercury. Because the γ -glutamyltransferase lines the canalicular portion of the plasma membrane of hepatocytes, it is likely that prevention of the degradation of GSH to which inorganic mercury is bound, would most likely prevent the entry of the mercuric ion into the hepatocyte, much like what occurs in the luminal compartment of the proximal tubule. It is less clear as to what mechanism is responsible for the enhanced hepatic accumulation of mercury in light of depletion of GSH. One possibility is that GSH plays a role as a ligand to conjugate with

mercuric ions within hepatocytes and that the formation of a mercuric-GSH conjugate is important in the extrusion or outward movement of mercury from the hepatocyte across the canalicular and/or sinusoidal membrane. Depletion of GSH in hepatocytes would then diminish the amount of mercuric ions that could be conjugated with GSH, and thus, could result in increased hepatocellular accumulation/retention of mercury. This hypothesis is supported, in part, by data linking the hepatocellular secretion of mercury and GSH into the bile (17, 18).

In conclusion, this study has demonstrated that depletion of intracellular GSH can alter significantly the accumulation of a nontoxic dose of inorganic mercury in the kidneys, liver, and blood. The mode by which GSH depletion was induced, however, played an important role on the influence exerted in the disposition of mercury. We suggest that whereas GSH or cysteine status by themselves have the potential to influence the uptake and accumulation of mercury, particularly in the kidneys, there are additional factors that affect the renal and hepatic handling of mercury. A critical difference between this study and some previous investigations (on the effects of GSH depletion on the transport and disposition of mercury) is that we used a nontoxic dose of inorganic mercury, whereas others have used moderately to highly nephrotoxic doses. We believe that this is the first study in which a nontoxic dose of inorganic mercury was used in the evaluation of GSH status on the renal and hepatic disposition of inorganic mercury.

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