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DEPENDENCE OF BILIARY SECRETION OF INORGANIC MERCURY ON THE BILIARY TRANSPORT OF GLUTATHIONE

NAZZARENO BALLATORI* and THOMAS W. CLARKSON

Division of Toxicology, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, Rochester, NY 14642, U.S.A.

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Abstract—The interrelation between the biliary transport of glutathione (GSH) and of inorganic mercury was investigated in suckling and adult male and female rats. The 14-day-old rat secreted inorganic mercury into bile at one-seventh the rate of the 28-day-old rat. Development of the ability to secrete mercury paralleled development of the ability to secrete GSH. The inability of the 14-day-old rat to secrete mercury and GSH occurred despite hepatic tissue concentrations of both of these compounds which were similar to those of adult rats. In adult rats, inhibition of GSH secretion by sulfobromophthalein (BSP) administration resulted in a parallel inhibition of mercury secretion. Conversely, the increase in the rate of GSH secretion into bile after cysteine or GSH administration was accompanied by an increase in the rate of mercury secretion into bile. The changes in the biliary secretion of mercury and of GSH after treatment with cysteine or GSH were not closely parallel, probably because of the tissue redistribution of mercury effected by these sulfhydryl-containing compounds. Mercury secretion into bile was independent of the changes in bile flow produced by dehydrocholate (DHC) or hypertonic sucrose, but it was closely related to the rate of GSH secretion. Further, sex differences and individual variability in the biliary secretion of inorganic mercury were correlated with differing abilities to secrete GSH into bile. These results suggest that the biliary secretion of inorganic mercury is in large part dependent on the biliary transport of GSH.

The previous report [1] has demonstrated that inorganic mercury in bile was bound predominantly to a substance of low molecular weight. This low molecular weight substance was tentatively identified as glutathione (GSH), suggesting a role of GSH in determining the biliary secretion of inorganic mercury.

A biliary transport system for GSH has been described recently [2, 3]. This transport system was shown to be the rate-limiting mechanism for the biliary secretion of the organomercurial methylmercury, and was speculated to play a role in the biliary secretion of all metals that have a selective affinity for reduced sulfhydryl groups [2, 3].

The present study was undertaken to determine the role of the GSH transport system in the regulation of the biliary secretion of inorganic mercury.

MATERIALS AND METHODS

Mercuric chloride was obtained from the Fisher Chemical Co., Fair Lawn, NJ, and $^{203}HgCl_2$ from the New England Nuclear Corp., Boston, MA. Sodium dehydrocholate (DHC), disodium ethylenediamine tetraacetic acid (EDTA), GSH and L-cysteine were obtained from the Sigma Chemical Co., St. Louis, MO, and sulfobromophthalein (BSP) was from the Aldrich Chemical Co., Milwaukee, WI.

Male (200–300 g), female (190–280 g) and lactating rats with pups (Sprague–Dawley) from Charles River Laboratories, Boston, MA, were fed *ad lib.* up to the time of the experiment. The trachea, jugular vein and bile duct were cannulated under sodium pentobarbital anesthesia (65 mg/kg for adult rats, 50 mg/kg for suckling rats, i.p.). Additional anesthetic was administered i.p. as required throughout the experiment. Body temperature was monitored by a Tele-Thermometer connected to a rectal probe (Yellow Springs Instrument Co., Yellow Springs, OH) and was kept at 37–38° by placing the animals on a support tray above a heated water bath. Mercuric chloride labeled with ^{203}Hg was injected into the jugular vein cannula at a dose of 0.2 mg Hg/kg body weight (1.0 μmole Hg/kg) in a solution of 0.9% NaCl.

For the adult animals, the volume of the mercury solution was 1 ml/kg, and the specific activity was 25 μCi/mg mercury. Bile was collected every 30 min for 5 hr into ice-chilled tared tubes containing 0.2 ml of 0.2 M EDTA. In the DHC experiments, the volume of EDTA was doubled. The compound to be tested, or saline for controls, was administered intravenously over a 30-min interval starting at 2 hr after the injection of mercury, in a total volume of 4 ml/kg, given as bolus injections of 0.57 ml/kg every 5 min. The doses of the various compounds, in mmoles/kg, were as follows: BSP, 0.044; GSH and cysteine, 1.0; DHC, 0.35; and sucrose, 8.0.

For the 14-, 21- and 28-day-old rats the volume of the mercury solution was 2 ml/kg and the specific activity was approximately 75 μCi/mg mercury. Bile was collected every hour for 4 hr into ice-chilled tared tubes containing 0.2 ml of 0.2 M EDTA. Each

* Author to whom all correspondence should be addressed.

age group had an equal number of male and female rats. All animals received 4 ml saline/kg intravenously over a 30-min interval starting at 2 hr after the injection of mercury.

At the end of the experiment, blood was collected from the abdominal aorta into a syringe containing approximately 10 μl of 0.2 M EDTA/ml of whole blood. The liver and kidneys were quickly excised and blotted. The left kidney and the median lobe of the liver were used for GSH analysis, while the right kidney and the left lateral lobe of the liver were used for mercury determination. The mercury content of liver, kidney, plasma, packed red blood cells and bile was determined by gamma scintillation counting in a Packard model 3002 scintillation spectrometer, at a counting efficiency of approximately 57%. The GSH content of liver, kidney and bile was estimated by measurement of nonprotein reduced sulfhydryl groups by the method of Sedlak and Lindsay [4]. Previous reports have shown that GSH is the most abundant nonprotein sulfhydryl-containing compound in tissues [5], and that GSH accounts for at least 90% of the reduced sulfhydryl groups in bile [6, 7]. Bile volume was determined gravimetrically assuming a density of 1.0 g/ml.

The data were compared by an analysis of variance. When the analysis indicated that a significant difference existed ($P < 0.05$), the means were compared by Student's *t*-test. Differences were considered to be statistically significant when $P < 0.01$. All values are expressed as mean ± standard error.

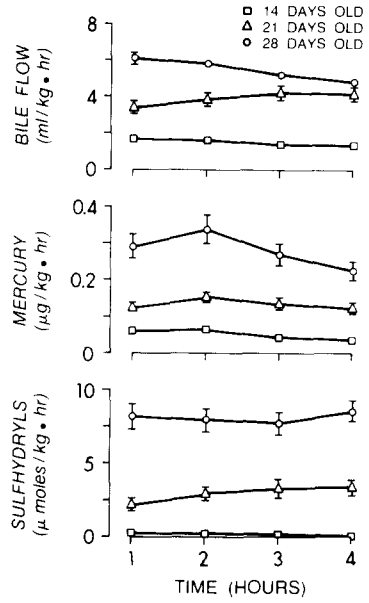

Fig. 1. Developmental changes in bile flow, mercury secretion into bile, and reduced sulfhydryl secretion into bile. An intravenous injection of mercury, 0.2 mg/kg, as $^{203}HgCl_2$ was given to 14-day-old (□; N = 14), 21-day-old (△; N = 12), and 28-day-old (○; N = 12) rats, and bile was collected every hour for 4 hr thereafter. Values are means ± S.E.

RESULTS

Developmental changes in the biliary secretion of inorganic mercury. The 14-day-old rat has been shown previously to lack the ability to secrete GSH into bile [2]. Figure 1 confirms this observation and further shows that the 14-day-old rat secreted inorganic mercury into bile at one-seventh the rate of the 28-day-old rat. This difference was only partly explained by the lower rate of bile formation in the 14-day-old; bile flow in the 14-day-old rat was approximately one-third that of the 28-day-old. Development of the ability to secrete mercury paralleled the development of the ability to secrete sulfhydryl groups.

The 14-day-old rat was unable to secrete sulfhydryl groups and mercury into bile despite hepatic concentrations of these compounds which were comparable to those of adult rats (Table 1). The hepatic concentration of sulfhydryl groups in the 14-day-old rat was slightly lower (22%) than that of the 28-day-old rat.

In agreement with the data of Jugo [8], the kidney mercury level in the suckling rat was significantly lower (Table 1). Interestingly, the concentration of reduced sulfhydryl groups in kidney tissue was also significantly lower in the 14-day-old rat.

No statistically significant sex-related difference in either mercury or sulfhydryl distribution or secretion was found in the 14-, 21- or 28-day-old rats.

Sex differences and individual variability in adult rats. As previously reported [3], adult female rats were found to secrete sulfhydryl groups into bile at a significantly higher rate than male rats (Fig. 2C). Female rats also secreted inorganic mercury into bile at a proportionally higher rate (50%) than male rats (Fig. 2B). Bile flow rates were similar (Fig. 2A), as were the liver concentrations of mercury (Table 2). However, the hepatic concentration of sulfhydryl groups was 21% higher in the female rat, which

Table 1. Tissue concentrations of mercury and nonprotein sulfhydryl groups in young rats*

Age (days)	N	Body wt (g)	Mercury (μg/g)				Sulfhydryls (μmoles/g)	
			Liver	Kidney	RBC	Plasma	Liver	Kidney
14	14	31.5 ±0.6	0.58 ± 0.03	2.29 ± 0.14†	0.75 ± 0.03†	0.181 ± 0.008	4.14 ± 0.18†	2.36 ± 0.09†
21	12	51.2 ± 1.6	0.58 ± 0.04	5.86 ± 0.26†	0.52 ± 0.03	0.151 ± 0.009	5.59 ± 0.27	3.18 ± 0.17†
28	12	86.8 ± 3.2	0.50 ± 0.02	8.19 ± 0.38	0.53 ± 0.05	0.166 ± 0.007	5.34 ± 0.35	4.03 ± 0.12

* Values are means ± S.E. The animals were killed 4 hr after the i.v. injection of 0.2 mg Hg/kg as $^{203}HgCl_2$, and the tissues were assayed for mercury and nonprotein sulfhydryl content.

† Significantly different from the 28-day-old rat ($P < 0.01$).

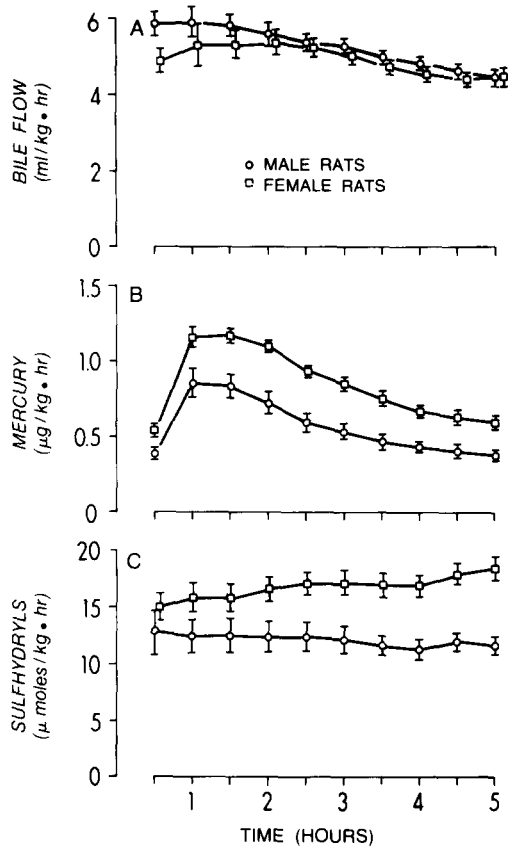

Fig. 2. Sex differences in bile flow (A), rate of mercury secretion (B), and rate of sulfhydryl groups secretion (C). Bile was collected for 5 hr, in 30-min intervals, after the i.v. administration of 0.2 mg Hg/kg as $^{203}HgCl_2$. Values are means ± S.E. of ten rats per group.

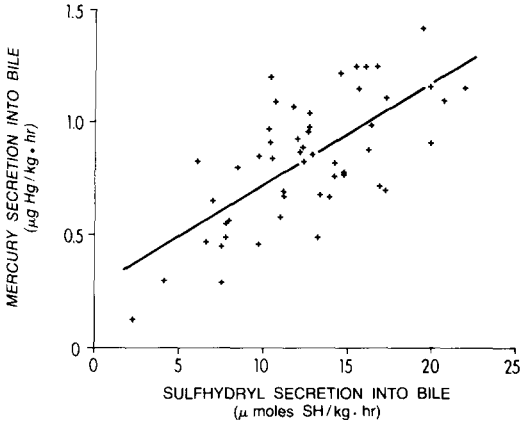

Fig. 3. Relation between the rates of secretion of mercury and reduced sulfhydryl groups into bile for fifty-two adult male rats. Each point denotes the rates of secretion of mercury and sulfhydryl groups into bile during the interval from 1.5 to 2.0 hr after the i.v. administration of 0.2 mg Hg/kg as $^{203}HgCl_2$. All of these animals had been treated identically up to and including 2 hr after dosing with mercury. The equation for the linear regression is $y = 0.045x + 0.268$, $r = 0.69$, $P < 0.001$.

Table 2. Tissue concentrations of mercury and nonprotein sulfhydryl groups in adult rats*

Sex	Treatment (N)	Body wt (g)	Mercury (μg/g)				Sulfhydryls (μmoles/g)	
			Liver	Kidney	RBC	Plasma	Liver	Kidney
Male	Saline (10)	260 ± 17	0.69 ± 0.04	8.36 ± 0.53	0.71 ± 0.06	0.203 ± 0.005	4.62 ± 0.28	3.22 ± 0.13
Female	Saline (10)	232 ± 8	0.72 ± 0.05	10.84 ± 0.55	0.52 ± 0.03	0.198 ± 0.007	5.57 ± 0.42†	4.13 ± 0.11†
Male	Glutathione (9)	240 ± 8	0.50 ± 0.03†	9.88 ± 0.44	0.66 ± 0.02	0.175 ± 0.005†	5.88 ± 0.20†	3.24 ± 0.05
Male	L-Cysteine (8)	262 ± 15	0.59 ± 0.06	11.20 ± 0.45†	0.64 ± 0.02	0.179 ± 0.007	5.96 ± 0.14†	3.17 ± 0.12
Male	Sulfobromophthalein (6)	232 ± 10	0.65 ± 0.03	8.05 ± 0.20	0.63 ± 0.04	0.205 ± 0.011	4.48 ± 0.36	3.51 ± 0.04
Male	Dehydrocholate (9)	231 ± 13	0.70 ± 0.08	7.81 ± 0.57	0.65 ± 0.06	0.197 ± 0.009	5.00 ± 0.30	3.00 ± 0.10
Male	Sucrose (6)	249 ± 20	0.72 ± 0.05	7.80 ± 0.42	0.66 ± 0.06	0.257 ± 0.023	4.58 ± 0.40	2.71 ± 0.14

* Values are means ± S.E. The various compounds were administered i.v. over a 30-min interval starting at 2 hr after the i.v. injection of 0.2 mg Hg/kg as $^{203}HgCl_2$. The animals were killed 5 hr after mercury administration.
† Significantly different from male controls ($P < 0.01$).

might explain, at least in part, the higher rate of secretion of sulfhydryl groups in the female rat.

Female rats also had significantly higher (28%) kidney levels of sulfhydryl groups (Table 2). Kidney mercury levels were also 30% higher in female rats; however, this difference was not statistically significant at the $P < 0.01$ level.

It has been reported previously that there are large individual differences in the biliary secretion of GSH [3]. In this study it was of interest to determine whether the individual differences in GSH secretion would correlate with individual differences in mercury secretion into bile. For this purpose the values for mercury and GSH secretion during the interval from 1.5 to 2.0 hr after mercury administration, of all male rats used in this study, were combined and plotted as shown in Fig. 3. The 1.5 to 2.0 hr interval was chosen since this is the interval immediately preceding treatment (i.e. treatment with BSP, GSH, DHC, etc.) and, therefore, all animals had been treated identically up to this time point, and because by 1.5 hr the organ distribution of mercury was essentially the same as that observed after 5 hr, so that distributional factors (e.g. hepatic uptake) were minimized. Figure 3 shows that there was a significant correlation between the ability of rats to secrete mercury and their ability to secrete sulfhydryl groups into bile.

Effects of BSP, cysteine and GSH administration. Inhibition of GSH secretion into bile by BSP [3] resulted in a concomitant decrease in the biliary secretion of mercury (Fig. 4). Inhibition of the secretion of both sulfhydryl groups and mercury was maximal during the first two collection periods after the administration of BSP, the period during which most (70%) of the BSP was secreted into bile. It was also during this time period that a small increase in bile flow was noted.

The close correlation between secretion of mercury and sulfhydryl groups, as described above, was not found when either cysteine or GSH was administered to animals (Fig. 4). Cysteine and GSH have been shown to be effective in increasing hepatic and biliary GSH levels [3]. GSH is not taken up intact by the liver but is rapidly cleared from the plasma (half-time < 2 min) and degraded to its constituent amino acids, mainly in the kidney [9]. A large fraction of the surplus plasma cysteine is rapidly (within minutes) incorporated into liver GSH, so that the plasma as well as the intracellular concentrations of free cysteine are kept quite low [10].

Both GSH and cysteine increased the rates of secretion of sulfhydryl groups to 140% of the pretreatment values at 1 hr (Fig. 4). The rates remained at this elevated level for the remainder of the experiment. Mercury secretion was elevated significantly for 2 hr after GSH or cysteine administration, but returned to control values in the last two collection periods. Cysteine produced a larger increase in the rate of mercury secretion into bile.

The lack of parallelism between the secretion of mercury and sulfhydryl groups after cysteine and GSH administration may be explained in large part by the changes in the distribution of mercury as well as sulfhydryl groups, effected by these compounds (Table 2). Cysteine and GSH administration pro-

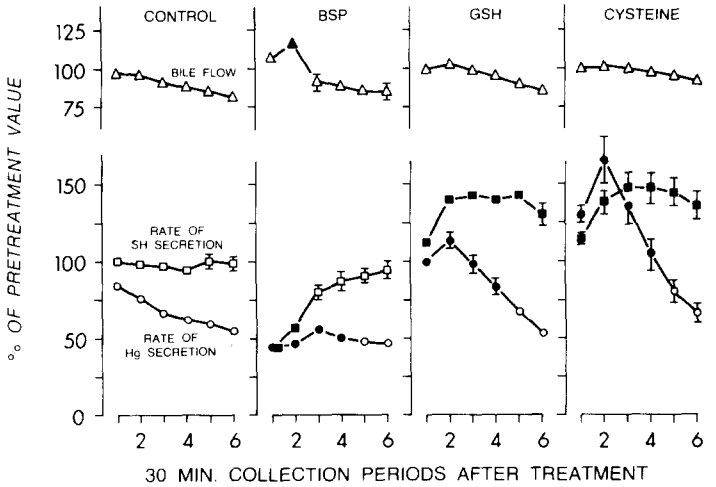

Fig. 4. Effects of sulfobromophthalein (BSP; 0.044 mmole/kg; N = 6), glutathione (GSH; 1.0 mmole/kg; N = 9), and cysteine (1.0 mmole/kg; N = 8) on bile flow rate and on the rates of secretion of mercury and reduced sulfhydryl groups. The compounds were administered i.v. over a 30-min interval starting at 2 hr after the i.v. administration of 0.2 mg Hg/kg as $^{203}HgCl_2$. Controls (N = 10) were given an equivalent volume of saline. The first 30-min collection period represents bile collected during the treatment period. Bile was collected for an additional 2.5 hr, in 30-min intervals, for a total of 5 hr from the initial administration of mercury. Solid symbols mark significant differences from control values ($P < 0.01$). The pretreatment (100%) values for bile flow (ml/kg·hr), rate of mercury secretion in bile (μg Hg/kg·hr), and rate of sulfhydryl groups secretion in bile (μmoles/kg·hr) were, respectively: controls, 5.58 ± 0.29, 0.72 ±0.07 and 12.4 ±1.3; BSP, 5.68 ± 0.16, 0.84 ± 0.14 and 11.7 ± 2.1; GSH, 5.77 ± 0.28, 0.92 ± 0.03 and 12.0 ± 0.8; and cysteine, 5.40 ± 0.26, 1.08 ± 0.06 and 14.5 ± 1.5. All values are means ± S.E.

duced significant increases in the hepatic concentration of sulfhydryl groups, and slight decreases in hepatic, plasma and red blood cell levels of mercury, as measured at the end of the experiment. Statistically significant changes were observed in the concentration of mercury in liver and plasma after GSH administration, and in renal mercury concentration after cysteine administration (Table 2).

Thus, the large increases in mercury secretion into bile brought about by cysteine and GSH administration (Fig. 4) are due to the combined effects of an increased mobilization of mercury to the liver, as well as to an increase in the rate of secretion of sulfhydryl groups into the bile. The decrease in the rate of mercury secretion to control levels during the last hour of the experiment (Fig. 4) is probably explained by the decrease in hepatic mercury concentrations seen at the end of the experiment (Table 2). While the rate of secretion of sulfhydryl groups continued at an elevated level for the entire experiment, the rate of mercury secretion did not keep up because the hepatic mercury pool was being depleted.

Effects of DHC and hypertonic sucrose. DHC produced a doubling of the rate of bile flow during the first collection period (Fig. 5). Bile flow fell rapidly to pretreatment values in the third and subsequent collection periods. The rates of mercury secretion, however, were approximately 35 and 31% lower than control animals during the first and second collection periods, respectively, after DHC infusion. The rates of secretion of sulfhydryl groups were also slightly decreased after DHC administration; a 24% decrease was observed in the second collection period. The rates of sulfhydryl groups and mercury secretion returned to control values at the same time as bile flow returned to normal.

Decreasing the rate of bile flow with hypertonic sucrose did not affect the net rates of secretion of either sulfhydryl groups or of mercury (Fig. 5). During the first two collection periods after sucrose administration bile flow was decreased approximately 25%, and there were proportional increases in the concentrations of sulfhydryl groups and mercury in bile.

Treatment with DHC or sucrose produced no significant changes in the concentrations of either mercury or sulfhydryl groups in any of the tissues analyzed (Table 2).

DISCUSSION

The results presented here show that the rate of inorganic mercury secretion into bile is closely coupled to the rate of GSH secretion. Ontogenic, sex, and individual differences in the rates of GSH secretion were correlated with differing rates of secretion of mercury into bile. Also, changes in the rate of GSH secretion induced by the exogenous agents BSP, cysteine, GSH, DHC and sucrose were closely followed by changes in the rate of mercury secretion. Thus, it appears that the previously described biliary transport system for GSH [2, 3] is an important determinant of the transport of inorganic mercury from liver cells into bile. While other intracellular mercury ligands, such as metallothionein,

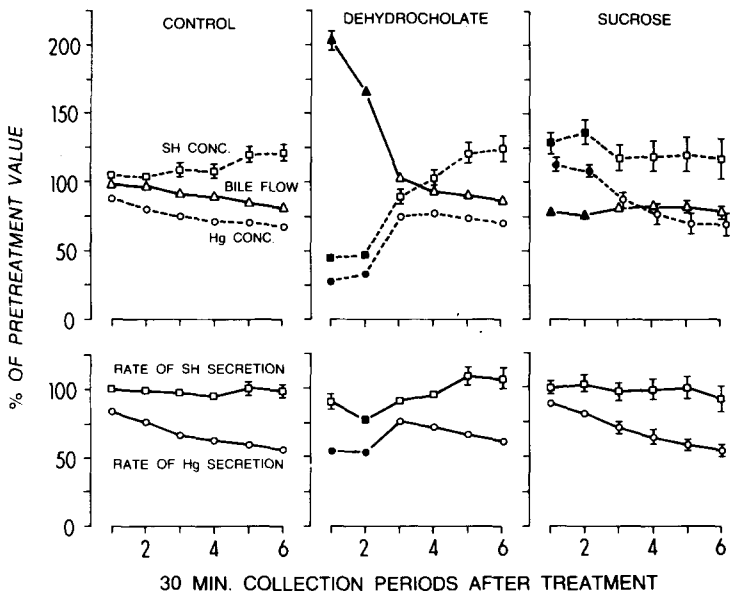

Fig. 5. Effects of dehydrocholate (0.35 mmole/kg; N = 9) and hypertonic sucrose (8.0 mmoles/kg; N = 6) on the rate of sulfhydryl groups secretion (□——□), rate of mercury secretion (○——○), bile flow (△——△), concentration of sulfhydryl groups in bile (□- - -□), and concentration of mercury in bile (○- - -○). The pretreatment (100%) values for bile flow (ml/kg·hr), rate of mercury secretion (μg Hg/kg·hr), and rate of sulfhydryl secretion in bile (μmoles/kg·hr) were, respectively: controls, 5.58 ± 0.29, 0.72 ± 0.07 and 12.4 ± 1.3; dehydrocholate, 6.30 ± 0.30, 0.85 ± 0.11 and 15.6 ± 1.5; and sucrose, 5.36 ± 0.27, 0.85 ± 0.07 and 10.9 ± 0.8. All values are means ±S.E. Explanatory information is the same as in the legend of Fig. 4.

have been shown to play important roles in sequestering mercury once it has reached the intracellular space, they do not affect the rate of uptake into the cell, and they most likely also do not affect the relative rate of metal efflux from the cells [11]. That is, these metal binding proteins are effective in binding and sequestering mercury; however, they appear to play only an indirect role in the actual transport of mercury across cell membranes. Indeed, metallothionein has not been found in bile [12].

The importance of GSH in determining the tissue distribution and uptake of mercury is becoming increasingly apparent. Mercury compounds have an exceptionally high affinity for sulfhydryl groups [13, 14]. GSH, the most abundant nonprotein sulfhydryl-containing compound in all tissues [5] and in bile [6, 7], readily complexes with mercury [13, 14]. Evidence is accumulating that the low molecular weight complexes thus formed play a significant role in the transport and tissue distribution of mercury compounds. Tissue GSH levels have been shown to be an important determinant of tissue mercury deposition [7, 15–19]. In addition, the distribution of mercury compounds can be altered by treatment with GSH or precursors of GSH (cysteine, *N*-acetylcysteine, methionine) ([3, 15, 17–22] and Table 2). Mercury–GSH complexes have been isolated from various tissues [6, 23–27]. Further, the transport of methylmercury [2, 3] and inorganic mercury (present study) into bile is determined in large part by the rate of GSH secretion into bile.

One of the more intriguing problems in heavy metal toxicology is the selective accumulation and toxicity of metals in the kidney. Recent evidence suggests that GSH is at least partially responsible for the observed phenomena. Richardson and Murphy [16] were able to demonstrate a high correlation between renal GSH levels and renal mercury accumulation. Johnson [28] showed that the GSH-induced alterations in the kidney uptake of mercury were correlated with the subsequent development of renal damage. Similarly, ontogenic changes in the sensitivity of the kidney to mercury-induced damage [29] may be related to the lower renal mercury concentrations ([8] and Table 1), which are in turn at least partially explained by the lower GSH levels in the kidneys of young animals (Table 1). The ontogenic changes in the GSH-metabolizing enzymes in the kidney, in particular γ-glutamyl transpeptidase [30], may be an equally important factor in the lower kidney uptake of mercury. Indeed, Alexander and Aaseth [18] recently have presented preliminary evidence for a relation between the inter-organ metabolism of GSH and the organ distribution of methylmercury.

GSH also appears to be involved in mediating some of the observed sex differences in the disposition of mercury compounds. Female rats secrete methylmercury [3] and inorganic mercury (Fig. 2), as well as GSH into bile at higher rates than male rats. It has been speculated that this difference may explain both the shorter biological half-time of methylmercury in female rats and why female rats are more sensitive to renal damage after administration of methylmercury [3].

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