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INHIBITION OF THE ENZYMES OF GLUTATHIONE METABOLISM BY MERCURIC CHLORIDE IN THE RAT KIDNEY: REVERSAL BY SELENIUM

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Abstract—The treatment of rats with 10 μmoles/kg (s.c.) of mercuric chloride (Hg^{2+}) caused time-dependent decreases in the activities of the enzymes of the glutathione (GSH) metabolism pathway in the kidney. Twenty-four hours after administration of Hg^{2+}, the activities of γ-glutamylcysteine synthetase and glutathione disulfide (GSSG)-reductase in the kidney were decreased by 50–60%, and the activities of the GSH catabolic enzymes, γ-glutamyl transpeptidase and GSH-peroxidase, were decreased by 25–35%. In the liver, only the activity of GSSG-reductase was decreased at this time. The observed decreases in the enzyme activities were not accompanied by a depression in the cellular protein concentration. The same pattern of enzyme response was noted when rats were given 30 μmoles/kg Hg^{2+}; however, the decreases in the specific activity of the enzymes were accompanied by great losses in the cellular protein concentrations in both the liver and the kidney (35–42%). This dose of Hg^{2+} also caused significant decreases in the concentration of GSH in both organs. *In vitro*, Hg^{2+} only inhibited the activity of GSSG-reductase. When rats were given sodium selenite (Na_2SeO_3; 5, 10 or 20 μmoles/kg, s.c.) 30 min after Hg^{2+} treatment (10 μmoles/kg), the Hg^{2+}-related depressions in the activities of the enzymes of GSH metabolism in the liver and the kidney were blocked. Also, in rats treated with 30 μmoles/kg Hg^{2+}, the administration of 10 μmoles/kg selenium significantly decreased the magnitude of depression in the concentration of GSH in the kidney.

Glutathione (GSH) is the major cellular thiol which binds electrophilic molecular species, free radical intermediates, and heavy metal ions such as mercury [1–5]. Early studies by Clarkson [5] implied the involvement of GSH in the *in vivo* binding of mercurials. Richardson and Murphy [6], utilizing labeled methylmercury chloride (CH_3HgCl), established the role of GSH in the *in vivo* binding of the metal ion. Recently, Eaton *et al.* [7] studied the effect of Hg^{2+} on cellular GSH levels and reported on the capacity of the metal ion to alter the concentration of GSH in the rat kidney. The pattern of alteration, however, was dependent on the dose of Hg^{2+}. This finding was consistent with the previous reports on the capacity of metal ions, such as Co^{2+} and Ni^{2+}, to alter the cellular levels of GSH in a biphasic manner, i.e. a depression followed by an increase [8–11].

In contrast to Hg^{2+}, selenium (Na_2SeO_3) has been shown to increase the concentration of GSH in rat liver [7, 12, 13]. The reported selenium-related increases in the cellular content of GSH appear to be a manifestation of the recently described ability of the element to increase the activities of γ-glutamylcysteine synthetase and glutathione disulfide (GSSG)-reductase [13]. Unlike GSH-peroxidase, which is a selenoenzyme [14, 15], γ-glutamylcysteine synthetase and the GSSG-reductase do not require selenium for activity. Therefore, the noted increases in the activities of γ-glutamylcysteine synthetase and GSSG-reductase do not reflect an increased availability of selenium for the formation of active enzyme–selenium complex. The activity of γ-glutamylcysteine synthetase is believed to be rate-limiting in the biosynthesis of GSH [16], and GSSG-reductase catalyzes the conversion of GSSG to GSH.

The ability of selenite and its compounds that are metabolized to selenite to modulate the adverse effects of mercurial compounds is well-established [17–21]. However, the mechanism of the protective effect of selenium is not fully understood. The present study was undertaken to investigate whether the treatment of rats with Hg^{2+} results in alterations in the activities of enzymes involved in GSH metabolism and to explore the possible modulation by selenium of the effects of Hg^{2+} on GSH metabolism. These studies were carried out using the kidney, a target organ for Hg^{2+}, and the liver which is not considered to be a target tissue.

MATERIALS AND METHODS

Male Sprague–Dawley rats (180–220 g) were purchased from Harlan Industries, Madison, WI. The animals were maintained on a 12-hr light and dark cycle and were given food and water *ad lib*. All injections were made between 8.00 and 9.00 a.m. Sodium selenite (Na_2SeO_3) and mercuric chloride ($HgCl_2$) were dissolved in saline and were injected subcutaneously (5.0 ml/kg). The control group received saline. The regimen of treatments is

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described in the legends to the tables and figure. Glutathione, GSSG, NADPH, ATP, *N*-ethylmaleimide (NEM), GSSG-reductase (yeast), glutamate, L-α-aminobutyrate, L-γ-glutamyl-*p*-nitroanilide, and *o*-phthalaldehyde were purchased from the Sigma Chemical Co., St. Louis, MO. Sodium selenite was purchased from J. T. Baker, Phillpsburg, N.J.

The animals were decapitated, and the organs were fully perfused *in situ* via the inferior vena cava with 0.9% NaCl. Blood samples were obtained by cardiac puncture immediately before killing the animals. The following procedure was used for the preparation of the cytosol fraction. The livers and kidneys were homogenized in 2 and 5 vol., respectively, of Tris–HCl buffer (0.01 M, pH 7.5) containing 0.25 M sucrose. The cellular homogenates were centrifuged at 10,000 *g* for 20 min, and the resulting supernatant fractions were centrifuged at 105,000 *g* for 1 hr. The activity of γ-glutamyl transpeptidase was measured in the cellular homogenates, and the 105,000 *g* supernatant fractions were used for the determination of the activities of GSSG-reductase, GSH-peroxidase, and γ-glutamylcysteine synthetase. Plasma samples were prepared by centrifuging blood at 5000 *g* for 5 min.

Tissue GSH concentrations were measured by a modification of the method described by Cohn and Lyle [22] using unperfused organs. The organs (one kidney and a lobe of liver, the same lobe for every experiment) were homogenized in 5 vol. of an extraction mixture of 10% trichloroacetic acid/1 mM Na_2 EDTA/0.01 N HCl (1/1/1, by vol.). Protein was removed by centrifugation at 5000 *g* for 10 min. One milliliter of 0.5 M Na_2HPO_4 and 100 μl of *o*-phthalaldehyde in methanol (1 mg/ml) were added to a 50-μl sample of the supernatant fraction. The GSH values were detected fluorometrically with an Aminco–Bowman spectrofluorometer, using GSH as the standard. The excitation and the emission wavelengths were 328 and 430 nm respectively. This method was found most suitable for the measurement of tissue levels of GSH, particularly in the kidney. Unlike 5′5′-dithiobis-2-nitrobenzoic acid (DTNB), *o*-phthalaldehyde specifically reacts with GSH to form a highly fluorescent product, and it does not bind with other soluble thiols which constitute 30–40% of the total soluble thiol content of the kidney [23]. The concentration of blood and plasma GSH was measured using the above procedure, except deproteinization was accomplished using metaphosphoric acid rather than trichloroacetic acid [24].

The activities of GSH-peroxidase and the GSSG-reductase assays were determined by measuring the disappearance of NADPH at 25° using a dual beam Varian spectrophotometer at 340 nm. The GSH-peroxidase assay was conducted by a modified coupling method of Paglia and Valentine [25] as described by Lawrence *et al.* [26]. An enzyme unit was defined as 1 nmole NADPH oxidized per mg protein per min. The assay procedure used for the determination of GSSG-reductase activity was based on the method described by Massey and Williams [27]. The assay medium (1.0 ml) contained enzyme sources (60–100 μg protein), Na_2 EDTA (3 mM), bovine serum albumin (2 mg), NADPH (0.1 mM), GSSG (3 mM), and potassium phosphate buffer (50 mM, pH 7.6). The reaction was started by the addition of GSSG. The blank assay system did not contain GSSG. An enzyme unit was defined as 1 nmole NADPH oxidized per mg protein per min.

The assay procedure described by Sekura and Meister [28] for the measurement of γ-glutamylcysteine synthetase activity was adapted for the present studies. The reaction mixtures (1.0 ml) contained ATP (5 mM), $MgCl_2$ (20 mM), L-glutamate (10 mM), L-α-aminobutyrate (10 mM), EDTA (2 mM), bovine serum albumin (0.05 mg) and Tris–HCl buffer (100 mM, pH 8.2). The reaction was initiated by the addition of the enzyme source (1–2 mg protein). L-α-Aminobutyrate was not added to the blank assay mixtures. The duration of incubation was 30 min at 37°. The reaction was terminated by the addition of 1 ml trichloroacetic acid (10%), and the protein was precipitated by centrifuging (5000 *g*, 10 min). The liberated phosphate (Pi) in the supernatant fraction was determined colorimetrically (720 nm) by the method of Taussky and Shorr [29]. An enzyme unit was defined as 1 nmole P_i released per mg protein per min.

The activity of γ-glutamyl transpeptidase was measured by a modification of the method described by Tate and Meister [30]. The assay system (1.0 ml) consisted of enzyme protein (kidney, 20–50 μg protein; liver, 1–2 mg protein), glycylglycine (20 mM), L-γ-glutamyl-*p*-nitroanilide (2.5 mM), Triton X-100 (1.0%), NaCl (75 mM), and Tris–HCl buffer (0.05 M, pH 8.2). The duration of incubation was 10 min at 25°. For the measurement of liver γ-glutamyl transpeptidase activity, the cellular homogenate was preincubated with deoxycholic acid (1.0%) for 15 min at 25° prior to use in the assay system. The reaction was initiated by the addition of L-γ-glutamyl-*p*-nitroanilide. The rate of release of *p*-nitroaniline was followed from an increase in absorption at 405 nm. An extinction coefficient of 9.9 mM^{-1} cm^{-1} was used for the measurement of the enzymatic activity. The enzyme activity is expressed as the micromoles or nanomoles of product formed per minute per milligram of kidney and liver protein respectively.

The *in vitro* effects of Hg^{2+} on GSH concentration in the liver and kidney 5000 *g* supernatant fraction and on authentic GSH preparations were carried out as follows. Mercuric chloride was dissolved in Tris–HCl buffer (10 mM, pH 7.5), and 10-μl aliquots were added to 100-μl fractions of tissue preparations prior to the beginning of GSH determination. The mixtures were incubated for 15 min at 37°.

The activity of microsomal heme oxygenase was measured as described previously [31]. Protein concentrations were measured by the method of Lowry *et al.* [32] with bovine serum albumin as the standard. All experiments were repeated three to six times with one rat per experiment, and the data were analyzed using Student's *t*-test. The data are presented as the mean ± S.D.

RESULTS

Effects of mercuric chloride treatment on the activities of the enzymes of GSH metabolism and GSH

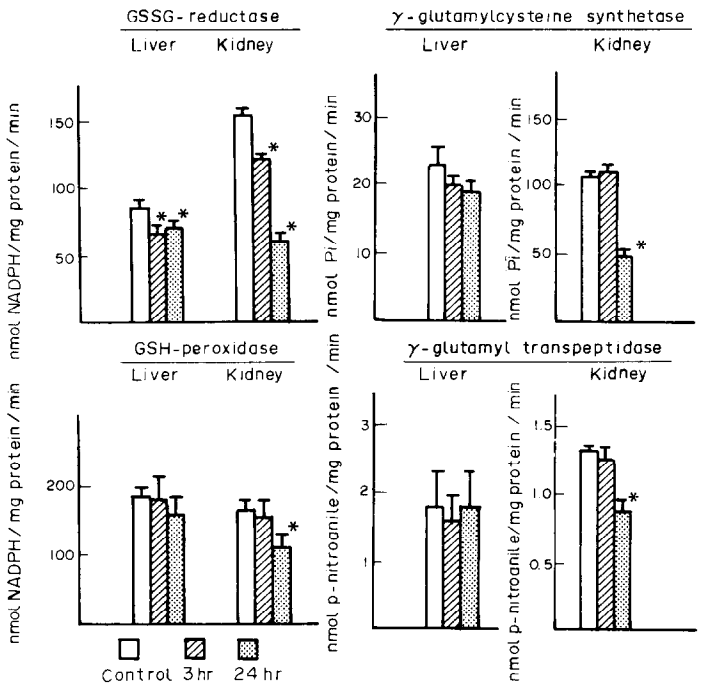

Fig. 1. Time-dependent *in vivo* effects of mercuric chloride on the activities of GSSG-reductase, γ-glutamylcysteine synthetase, γ-glutamyl transpeptidase and GSH-peroxidase in the rat liver and kidney. Groups of four rats (180–220 g) were treated with Hg^{2+} (10 μmoles/kg, s.c.) 3 and 24 hr before killing. The control rats received saline. The perfused liver and the kidney cytosol fractions were prepared and used for the measurement of the activities of GSSG reductase, γ-glutamylcysteine synthetase, and GSH-peroxidase. The activity of γ-glutamyl transpeptidase was measured in whole homogenate. The procedures employed for the measurements are detailed in Materials and Methods. The asterisk (*) indicates $P \leq 0.05$ when compared to the control values.

level in the rat liver and kidney. The effects of Hg^{2+} (10 μmoles/kg, s.c.) on the activities of γ-glutamylcysteine synthetase, GSSG-reductase, γ-glutamyl transpeptidase and GSH-peroxidase were investigated 3 hr and 24 hr after treatment (Fig. 1). Since hepatic GSH concentrations were the same in rats killed at 8.30 and 11.30 a.m., the same group of animals was used as controls for both 3-hr and 24-hr experiments. Also, at these times no diurnal variations in the activities of the enzymes of GSH metabolism were detected.

As shown in Fig. 1, the activities of hepatic and renal GSSG-reductases were decreased 3 hr after the administration of Hg^{2+}; no significant alterations in the activities of other enzymes of GSH metabolism were observed. When measured 24 hr after Hg^{2+} treatment, significant decreases in the activities of the γ-glutamylcysteine synthetase, GSSG-reductase, GSH-peroxidase, and γ-glutamyl transpeptidase in the kidney were noted. In the liver, the activity of GSSG-reductase was decreased, but the activities of the other enzymes measured remained unaltered.

In contrast to the potent inhibitory effect of Hg^{2+} on the activities of γ-glutamylcysteine synthetase and GSSG-reductase in the kidney, Hg^{2+} did not decrease renal GSH concentrations. Rather, as shown in Table 1, the plasma and renal concentrations of GSH were increased by Hg^{2+} treatment. On the other hand, the GSH content in the liver was decreased after giving Hg^{2+}. The elevation of GSH concentrations in the kidney may reflect the inhibitory action of Hg^{2+} on the activity of γ-glutamyl transpeptidase and the increased plasma level of GSH. In Hg^{2+}-treated rats, the plasma concentration of GSH was increased when compared to control rats.

The possibility that the observed inhibitory effects of Hg^{2+} on the enzymes of GSH metabolism may represent a generalized inhibition of cellular activities was explored. This was tested by measuring the activity of an enzyme not involved in GSH metabolism, e.g. the microsomal heme oxygenase. The activity of the heme catabolic enzyme was increased significantly rather than decreased by Hg^{2+} treatment. The liver and kidney microsomal heme oxy-

Table 1. *In vivo* effect of mercuric chloride on GSH concentration in the rat kidney, liver and plasma*

Treatment	GSH (mM)		
	Liver	Kidney	Plasma
Control	7.2 ± 0.3	1.91 ± 0.08	0.12 ± 0.01
Hg^{2+}	5.0 ± 0.7	3.16 ± 0.14†	0.18 ± 0.01†

* Groups of four rats were treated with Hg^{2+} (10 μmoles/kg, s.c.) or saline and were killed 24 hr later. Blood samples were obtained by cardiac puncture with heparinized syringes immediately before killing the animals. The concentration of GSH was determined as detailed in the Materials and Methods. The values are means ± S.D.

† $P \leq 0.05$ when compared to the control values.

genase activities 24 hr after the administration of Hg^{2+} were 4.5 ± 0.7 nmoles bilirubin per mg protein per hr and 2.6 ± 0.5 nmoles bilirubin per mg protein per hr respectively. The mean enzyme activities of the livers and the kidneys of the control animals were 1.3 ± 0.2 nmoles bilirubin per mg protein per hr and 0.8 ± 0.3 nmoles bilirubin per mg protein per hr respectively. The dose of Hg^{2+} employed in these studies did not alter the concentrations of protein in the cytosol fractions of the liver and the kidney at 3 and 24 hr (data not shown).

The inhibitory effects of Hg^{2+} on the specific activities of the enzymes of GSH metabolism were not further augmented by increasing the dose of the metal ion. As shown in Table 2, in the kidney 24 hr after treatment of rats with 30 μmoles/kg Hg^{2+} the specific activities of GSSG-reductase, γ-glutamylcysteine synthetase, γ-glutamyl transpeptidase and GSH-peroxidase were decreased essentially to the same extent as with 10 μmoles/kg of the metal ion (Fig. 1). Similarly, in the liver the specific activity of GSSG-reductase was decreased, with the activities of γ-glutamylcysteine synthetase and GSH-peroxidase remaining refractory to Hg^{2+} treatment. However, when expressed per gram of tissue, the activities of all GSH enzymes were markedly reduced in both organs. This finding suggests the loss of enzyme protein from the liver and kidney.

Effect of sodium selenite on the inhibition by mercuric chloride of the activities of GSH-metabolism enzymes and the decrease in the concentrations of GSH in the liver and kidney by mercuric chloride. The possible reversal by selenium of the Hg^{2+}-related inhibition of the activities of the enzymes of GSH metabolism in the liver and the kidney was investigated. The animals received sodium selenite (5, 10 and 20 μmoles/kg, s.c.) 30 min following Hg^{2+} treatment (10 μmoles/kg, s.c.) and were killed 24 hr after the last injection. Table 3 depicts interactions between selenium and Hg^{2+} on the activities of the enzymes of GSH metabolism. As shown, the treatment of rats with 5 μmoles/kg selenium was effective in protecting against the inhibitory effects of Hg^{2+} on the activity of GSSG-reductase in the liver as well as the activities of GSSG-reductase and γ-glutamylcysteine synthetase in the kidney. The administration of 10 μmoles/kg selenium to rats treated with Hg^{2+} fully blocked the deleterious effects of the metal on GSH metabolism enzymes in the liver and kidney. When the molar ratio of selenium/Hg^{2+} was increased to 2:1, the activities of GSSG-reductase and γ-glutamylcysteine synthetase in the liver were increased when compared to the control animals. Moreover, in the liver of animals treated with 10 or 20 μmoles/kg selenium alone, the activities of the enzymes of GSH biosynthesis were increased. The concentrations of protein in the liver and the kidney cytosol fractions were not altered by the regimen of treatments employed (data not shown).

The effects of 30 μmoles/kg Hg^{2+} on cellular GSH concentration and the possible interaction between selenium and Hg^{2+} on this variable were investigated. As shown in Table 4, the treatment of rats with 30 μmoles/kg Hg^{2+} decreased GSH levels in the liver and kidney. The administration of 10 μmoles/kg selenium prevented the depletion of GSH concen-

Table 2. *In vivo* effects of 30 μmoles/kg mercuric chloride on the activities of GSSG-reductase, γ-glutamylcysteine synthetase, γ-glutamyl transpeptidase and GSH-peroxidase in the rat liver and kidney*

Organ	Treatment	GSSG-reductase		γ-Glutamylcysteine synthetase		GSH-peroxidase		γ-Glutamyl transpeptides		Cytosol protein
		(E.U./mg protein)†	(E.U. × 10^3/g tissue)†	(E.U./mg protein)‡	(E.U. × 10^3/g tissue)‡	(E.U./mg protein)†	(E.U. × 10^3/g tissue)†	E.U./mg protein)§	(E.U./g tissue)§	(mg/g tissue)
Liver	Control	86 ± 5	5.20 ± 0.29	22.5 ± 3.3	1.26 ± 0.10	210 ± 15	12.07 ± 0.90			62 ± 6
	Hg^{2+}	63 ± 8‖	2.60 ± 0.30‖	21.5 ± 4.8	0.83 ± 0.09‖	198 ± 28	8.77 ± 1.01‖			41 ± 9‖
		(73)	(50)	(96)	(66)	(94)	(72)			(66)
Kidney	Control	140 ± 10	7.21 ± 0.62	145 ± 10	7.52 ± 0.62	154 ± 11	8.01 ± 0.39	1.56 ± 0.08	232 ± 14	52 ± 3
	Hg^{2+}	54 ± 10‖	1.66 ± 0.30‖	98 ± 7‖	2.84 ± 0.35‖	98 ± 7‖	3.52 ± 0.30‖	0.88 ± 0.16‖	120 ± 28	30 ± 1‖
		(38)	(23)	(67)	(38)	(64)	(44)	(56)	(52)	(58)

* Groups of six male Sprague–Dawley rats (180–220 g) were treated with Hg^{2+} (30 μmoles/kg, s.c.) or saline. The animals were killed 24 hr later; the liver and kidney homogenates and cytosol fractions were prepared and utilized for the above indicated measurements. Various determinations were carried out as described in Materials and Methods. The data presented are the means ± S.D. The data in parentheses represent the means expressed as the per cent of the control values.

† E.U. is defined as 1 nmole NADPH oxidized/min.

‡ E.U. is defined as 1 nmole P_i produced/min.

§ E.U. is defined as 1 μmole *p*-nitroaniline produced/min in the kidney; the enzyme activity in the liver was not measured.

‖ $P \leq 0.05$ when compared to the control values.

Table 3. Inhibition by sodium selenite of mercuric chloride-related decreases in the activities of GSSG-reductase, γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase in the rat liver and kidney*

Treatment (μmoles/kg)		GSSG-reductase (E.U./mg protein)†		γ-Glutamylcysteine synthetase (E.U./mg protein)‡		γ-Glutamyl transpeptidase (E.U./mg protein)§	
Hg^{2+}	selenium	Liver	Kidney	Liver	Kidney	Liver	Kidney
0	0	82 ± 6	129 ± 7	22.2 ± 1.9	127 ± 6	1.72 ± 0.39	1.35 ± 0.06
10	0	70 ± 4‖	76 ± 10‖	20.4 ± 1.4	59 ± 10‖	1.74 ± 0.48	0.92 ± 0.07‖
10	5	87 ± 5	123 ± 12	25.9 ± 2.1	127 ± 20	2.02 ± 0.29	0.99 ± 0.04‖
0	5	92 ± 7	132 ± 2	24.0 ± 1.6	121 ± 9	2.27 ± 0.41	1.32 ± 0.09
10	10	80 ± 11	120 ± 2	23.9 ± 4.5	131 ± 3	2.11 ± 0.31	1.43 ± 0.09
0	10	110 ± 7‖	122 ± 6	31.7 ± 1.7‖	133 ± 7	1.16 ± 0.41	1.28 ± 0.05
10	20	129 ± 6‖	133 ± 11	33.7 ± 1.7‖	125 ± 10	1.60 ± 0.29	1.29 ± 0.08
0	20	151 ± 6‖	144 ± 18	48.2 ± 1.2‖	137 ± 15	1.92 ± 0.52	1.27 ± 0.11

* Groups of four rats (180–220 g) were used for each treatment. The animals were treated with Hg^{2+} (10 μmoles/kg, s.c.) and after 30 min were given the indicated doses of selenium (s.c.). The control animals received saline. Twenty-four hours after Hg^{2+} treatment the animals were killed, and the liver and the kidney cytosol fractions were prepared. The above indicated measurements were conducted employing the precedures detailed in Materials and Methods.

† E.U. is defined as 1 nmole NADPH oxidized/min.

‡ E.U. is defined as 1 nmole P_i produced/min.

§ E.U. is defined 1 μmole *p*-nitroaniline produced/min in the kidney, and 1 nmole/P_i produced/min in the liver.

‖ $P \leq 0.05$ when compared to the control values.

tration by Hg^{2+} in the kidney and decreased the extent of the depleting effect of Hg^{2+} on the liver GSH concentration. In addition, the administration of 10 μmoles/kg selenium to rats treated with 10 μmoles/kg Hg^{2+} fully blocked the depletion of GSH level of Hg^{2+} in the liver (data not shown).

In vitro *studies. In vitro* effects of Hg^{2+} on the activities of GSSG-reductase, γ-glutamylcysteine synthetase, γ-glutamyl transpeptidase, and the cellular concentration of GSH were investigated. Also, the effects of selenium plus Hg^{2+} on the activities of these enzymes were explored. These studies were conducted using kidney preparations. As shown in Table 5, *in vitro* Hg^{2+} inhibited the activity of GSSG-reductase in a dose-dependent manner. Conversely, selenium at all concentrations used was ineffective in altering the activity of the enzyme, and the addition of selenium in combination with Hg^{2+} to the assay system did not provide protection against the inhibitory effect of Hg^{2+} on GSSG-reductase.

In contrast to the effect of Hg^{2+} on GSSG-reductase, when Hg^{2+} was added in concentrations up to 10 μM to the γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase assay systems, the element was essentially ineffective in altering the activities of the enzymes to a significant extent. Similarly, the additions of selenium, and selenium plus Hg^{2+} to the assay systems proved ineffective in altering the activities of the enzymes.

The effects of Hg^{2+} on renal and hepatic GSH concentrations are shown in Table 6. Various concentrations of Hg^{2+} were incubated (10 min, 37°) with the liver and the kidney homogenates and with authentic GSH solutions. The addition of Hg^{2+} to the tissue preparations decreases GSH levels in a concentration-dependent manner. However, Hg^{2+} was less effective in decreasing GSH concentration in the kidney preparation than in the liver homogenate. This difference may reflect binding of Hg^{2+} to available binding sites other than a GSH, in the kidney [23]. As shown, only minute concentrations of Hg^{2+} were required for marked decreases of GSH concentration when authentic preparation of the tripeptide was used.

Table 4. Effect of treatment with a combination of sodium selenite and mercuric chloride on the concentration of GSH in the rat liver and kidney*

Treatment	GSH (mM)	
	Liver	Kidney
Control	7.0 ± 0.2	2.01 ± 0.18
Hg^{2+}	5.1 ± 0.1†	1.32 ± 0.12†
Selenium	8.5 ± 0.7†	2.03 ± 0.15
Hg^{2+} + selenium	5.6 ± 0.3†	2.04 ± 0.27

* Groups of four rats were treated with 30 μmoles/kg Hg^{2+} (s.c.) and after 30 min were given 10 μmoles/kg selenium. The control animals received saline. Twenty-four hours later the rats were killed, and the tissue homogenates were prepared and used for GSH determinations as described in Materials and Methods.

† $P \leq 0.05$ when compared to the control values.

DISCUSSION

The data presented in this report describe the ability of inorganic mercury to inhibit the activities of GSH-metabolizing enzymes in the kidney, which is considered a target organ for Hg^{2+} toxicity. Mercury (10 μmoles/kg, 24 hr) inhibited the activity of γ-glutamylcysteine synthetase, the rate-limiting enzyme in GSH biosynthesis, and GSSG-reductase, as well as γ-glutamyl transpeptidase, the first enzyme in the degradation of GSH [30, 33], and GSH-per-

Table 5. *In vitro* effects of mercuric chloride and sodium selenite on the activities of GSSG-reductase, γ-glutamylcysteine synthetase and γ-glutamyl transpeptides in the kidney*

Additions		Enzyme		
Hg^{2+} (μM)	Sodium selenite (μM)	GSSG-reductase (% of control)	γ-Glutamylcysteine synthetase (% of control)	γ-Glutamyl transpeptidase (% of control)
0	0	100	100	100
0.1	0	88	101	99
0	0.1	100	99	100
0.1	0.1	88	101	100
1.0	0	14	97	99
0	1.0	100	99	99
1.0	1.0	14	98	98
1.0	5.0	14	101	102
0	10.0	100	101	103
0	100.0	99	99	102
10.0	0	0	98	102

* Mercuric chloride (Hg^{2+}) and sodium selenite were dissolved in Tris–HCl buffer (10 mM, pH 7.5). Ten microliter portions of the solutions were added to the assay systems at the beginning of the incubation period to obtain the desired concentrations of the elements. The activities of the enzymes were measured as described in Materials and Methods. The data are the average of two determinations.

The control values were: GSSG-reductase, 129 ± 7 nmoles NADPH oxidized per mg protein per min; γ-glutamylcysteine synthetase, 138 ± 6 nmoles P_i produced per mg protein per min; and γ-glutamyl transpeptidase, 1.35 ± 0.06 μmoles *p*-nitroaniline produced per mg protein per min.

oxidase. In the liver, only the activity of GSSG-reductase, which catalyzes the conversion of GSSG to GSH, was inhibited by Hg^{2+}.

In rats treated with a higher dose of Hg^{2+} (30 μmoles/kg), a marked depletion in the cellular concentration of GSH was observed in the liver and kidney. It appears that the Hg^{2+}-related decreases in the liver and kidney GSH concentrations (Table 4) were direct manifestations of depressions in the biosynthesis of GSH, although the direct interaction of Hg^{2+} with GSH also could have contributed to the depletion of tissue GSH content after Hg^{2+} treatment. Moreover, the possibility of altered levels of cellular GSSG and mixed disulfides in response to Hg^{2+} treatment cannot be ruled out. It is known that an increase in the concentrations of these two variables can result in a decreased cellular GSH concentration [34].

Table 6. *In vitro* effect of mercuric chloride on GSH concentrations in the liver and kidney preparations and authentic GSH*

Conc of Hg^{2+} (mM)	GSH source (% of control)		
	Liver	Kidney	Authentic GSH
0	100	100	100
0.5	67	74	40
1.0	24	55	5
2.0	23	42	0
4.0	16	39	0
8.0	5	25	0
10	3	19	0

* Liver and kidney homogenates were prepared from the organs of untreated animals. The 5000 g supernatant fraction was used for GSH determination. The procedure used for the tissue preparations and the assay of GSH are described in detail in Materials and Methods. The control concentrations of GSH were: liver, 3.0 mM; kidney, 2.0 mM; and authentic GSH, 2.0 mM.

In contrast to the liver, the lower dose of Hg^{2+} (10 μmoles/kg) increased cellular GSH concentrations in the kidney (Table 1). This observation may reflect the inhibition by Hg^{2+} of γ-glutamyl transpeptidase, which is highly active in the kidney, and of GSH-peroxidase. Moreover, there is a distinct possibility that increased kidney GSH levels may be related to the tissue uptake of extracellular GSH. Recently it has been shown that GSH may be released from the liver to the plasma and subsequently taken up by the kidney [35]. The present observation (Table 1) that the increased GSH concentrations in kidney and plasma were accompanied by a decreased GSH concentration in the liver is consistent with this mechanism. Other factors which may contribute to the ability of 10 μmoles/kg Hg^{2+} to decrease GSH concentration in the kidney include the preponderance of competing binding sites for Hg^{2+} ([23], Table 6), plus the fact that the activity of the γ-glutamylcysteine synthetase in the kidney was 5- to 6-fold greater than that in the liver (Tables 2 and 3).

Depression produced by Hg^{2+} (10 μmoles/kg) of the activities of γ-glutamylcysteine synthetase and GSSG-reductase in the kidney was particularly notable (Fig. 1). The marked difference in the extent of inhibitory effects of Hg^{2+} on the activities of these enzymes in the kidney in comparison to the liver may represent the accumulation and increased concentration of Hg^{2+} in the kidney, rather than differences in the properties of GSSG-reductase and γ-glutamylcysteine synthetase in the two organs.

Since GSSG-reductase is a disulfide enzyme [36], it is not surprising that Hg^{2+}, both *in vivo* (Fig. 1) and *in vitro* (Table 5), inhibited the activity of GSSG-reductase. The *in vivo* observation may also

involve other components, such as the inhibitor by Hg^{2+} of the synthesis or increased rate of degradation of the GSSG-reductase enzyme protein or both. Another possible mechanism for the marked depression of GSSG-reductase activity in the kidney is the loss of enzyme protein. Glutathione reductase is a soluble enzyme and the nephrotoxic effects of Hg^{2+} are well known; therefore, it could be speculated that the observed decrease in the activity of GSSG-reductase in the kidney might be related to a loss of the enzyme protein from the organ. However, unless a selective loss of GSSG-reductase was caused by 10 μmoles/kg Hg^{2+}, the contribution of this factor to the decreased activity of the enzyme could not be significant. The lack of effect of low concentrations of Hg^{2+} on cellular protein concentration is consistent with a direct action of the metal ion on the activity and/or turnover of the enzyme protein, rather than a loss of enzyme protein.

As shown in Table 2, however, the treatment of rats with 30 μmoles/kg Hg^{2+} led to the loss of nearly 35–40% of total cytosol protein from the liver and the kidney. This finding suggests the loss of enzyme protein from the liver and kidney as the cause of marked reduction in the activities of all GSH biosynthesis enzymes measured in this study in response to 30 μmoles/kg Hg^{2+}. It appears that the loss of enzyme protein from the liver cells was essentially the only cause of the decrease in the activities of GSH-peroxidase and γ-glutamylcysteine synthetase per gram tissue, whereas with GSSG-reductase the inhibition of enzyme activity, as well as the loss of enzyme protein, led to the severe decrease in the activity of the enzyme per gram of liver. In the kidney, however, with all enzymes studied a combination of both factors led to the severe depression in the activities of the enzymes per gram of tissue. The presently noted massive loss of soluble cellular protein is consistent with the finding of Cuppage and Tate [37] of the occurrence of marked proteinuria in rats 12–48 hr after Hg^{2+} treatment.

Unlike GSSG-reductase, γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase are not thiol-dependent enzymes and are not inhibited *in vitro* by Hg^{2+} (Table 5). It follows that the observed *in vivo* depressions in the activities of these enzymes caused by a low level of Hg^{2+} may also involve altered turnover rates of the proteins. *In vitro* studies have shown that Hg^{2+} can interfere with protein synthesis by inhibiting DNA-dependent RNA polymerases as well as by direct interaction with the nuclear material [38, 39].

The precise mechanism by which selenium protects against Hg^{2+} effects on the enzymes of GSH metabolism is not clear. However, there is a distant possibility that selenium-related increases in the activities of the enzymes of GSH metabolism may have contributed significantly to the biological inactivation of Hg^{2+}. Braun *et al.* [40] have demonstrated that urinary γ-glutamyl transpeptidase activity is a measure of kidney tubular lesions and observed that the enzyme activity in urine was increased markedly 24 hr after a single injection of Hg^{2+}. Further studies by Sener *et al.* [41] have shown that giving selenite to rats treated with Hg^{2+} decreased the excretion of γ-glutamyl transpeptidase in the urine. In the present study, it was noted that the activity of γ-glutamyl transpeptidase in the kidney was decreased by Hg^{2+}, and the administration of selenium reversed the decreased of enzyme activity caused by Hg^{2+} (Table 3). Groth *et al.* [42] demonstrated that, after long-term feeding experiments, Hg^{2+} and selenium are found in equimolar amounts in macrophages in nervous tissues. Koeman [43] and Kosta *et al.* [44] have reported on the accumulation of these elements in the brain and the liver of humans and other mammals with an atomic ratio of one. According to these findings, there is a possibility that the direct selenium Hg^{2+} interaction or their common affinity for -SH groups of tissue proteins may have been involved in the presently observed protective action of selenium against Hg^{2+}. However, it appears that such interactions could not constitute the sole mechanism for the observed blockade by selenium of the inhibitory effects of Hg^{2+} on the enzymes of GSH biosynthesis. As shown in Table 3, a 1:2 ratio of selenium/Hg^{2+} was very effective in preventing Hg^{2+} effects on enzymes of GSH biosynthesis. Moreover, the failure of selenium *in vitro* to prevent the inhibition of GSSG-reductase activity by Hg^{2+} is indicative of the involvement of mechanisms other than a direct interaction between selenium and Hg^{2+} in the *in vivo* protection offered by selenium against inhibition of GSSG-reductase activity by Hg^{2+}. Also, the regimen of treatments used in this study, i.e. Hg^{2+} treatment followed in 30 min by selenium does not favor the ready interaction of selenium and Hg^{2+}.

In conclusion, it is conceivable that the observed inhibitory effects of Hg^{2+} on the enzymes of GSH metabolism are involved in the general cellular toxicity of Hg^{2+}. It is also possible that the suppressive effects of selenium on Hg^{2+}-related inhibition of the enzymes of the GSH metabolism pathway may play a role in the known protective effects of selenium toward certain deleterious effects of Hg^{2+} toxicity.

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