Biochemical Pharmacology, Vol. 30, No. 23, pp. 3217–3223, 1981.
Printed in Great Britain.

0006-2952/81/233217-07 $02.00/0

EFFECT OF SELENIUM ON GLUTATHIONE METABOLISM

INDUCTION OF γ-GLUTAMYLCYSTEINE SYNTHETASE AND GLUTATHIONE REDUCTASE IN THE RAT LIVER

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(*Received* 29 *January* 1981; *accepted* 27 *April* 1981)

Abstract—The effect of sodium selenite (Na_2SeO_3, Se) on cellular glutathione metabolism was examined, particularly with respect to its ability to alter the activities of γ-glutamylcysteine synthetase and glutathione disulfide (GSSG) reductase. The treatment of rats with Se (5, 10 and 20 μmoles/kg) caused time- and dose-dependent increases in the activities of the synthetase and the reductase in the liver. The activity of γ-glutamylcysteine synthetase, the rate-limiting enzyme of the glutathione (GSH) biosynthesis, was particularly susceptible to Se treatment. The Se-mediated increases in the activities of the above enzymes were inhibited by puromycin and the increases could not be elicited *in vitro*. Selenium treatment caused time-dependent perturbations in the levels and ratio of GSSG and GSH in the liver. When compared to the control animals, rats treated for 3 hr with 10 and 20 μmoles Se/kg showed increased cellular levels of GSSG; in contrast, 24 hr after Se treatment the concentration of GSH was increased significantly. The activity of γ-glutamyl transpeptidase, which catalyzes the initial reaction in GSH breakdown, was unaltered by Se treatment. Repeated administration of low doses of Se (7.0 μmoles/kg, three times) also increased the activities of the reductase and the synthetase as well as the cellular levels of hepatic GSH and GSSG. It is suggested that the Se-mediated increases in the activities of γ-glutamylcysteine synthetase and GSSG-reductase represent cellular responses to Se-mediated perturbations in the levels and ratio of GSH and GSSG.

Previous reports have demonstrated the ability of certain metal ions such as Co^{2+}, Ni^{2+} and Hg^{2+} to alter the cellular content of glutathione (GSH) in the rat liver [1–4] and in the cultured avian liver cells [5]. In addition, Dalvi and Robbins [6] and Eaton *et al.* [4] reported recently the increase in cellular GSH levels after giving Se. The tripeptide GSH (L-γ-glutamyl-L-cysteinylglycine) is the immediate substrate for GSH-transferases and GSH-peroxidase, and constitutes the bulk of available sulfhydryl groups for binding and inactivation of electrophilic species [7, 8]. Moreover, GSH is a substrate for γ-glutamyl transpeptidase, a membrane-bound enzyme which catalyzes the initial reaction in the catabolism of the tripeptide [9, 10].

The pioneer studies by Rotruck *et al.* [11] and Flohe *et al.* [12] have established the central role of Se in GSH metabolism and its function as the integral moiety of the selenoenzyme, GSH-peroxidase. Subsequent studies have led to the development of a vast body of literature on various aspects of interrelationship between Se and GSH-peroxidase activity [13–17]. In the present study we report on the novel effects of Se on γ-glutamylcysteine synthetase and glutathione disulfide (GSSG)-reductase in the rat liver. This study also reports on marked perturbations in the cellular levels of both GSSG and GSH in the liver in response to treatment of rats with Se. Of the two enzymes affected by Se, γ-glutamylcysteine synthetase is believed to constitute the rate-limiting enzyme in the pathway of GSH biosynthesis [18], and GSSG-reductase is known to catalyze the reduction of GSSG to GSH.

MATERIALS AND METHODS

Male Sprague–Dawley rats (200–230 g) were used. The animals were allowed access to food and water *ad lib*. The animals were maintained on a 12-hr light (7:00 a.m.–7:00 p.m.) and dark cycle. Sodium selenite (Na_2SeO_3) was dissolved in saline, the pH was adjusted to 7.4, and the solution was injected by subcutaneous route. The treatment regimens are given in the legends of the appropriate tables and figures. The control group received saline. All injections were made between 8:30 and 9:00 a.m. Glutathione, GSSG, NADPH, ATP, *N*-ethylmaleimide (NEM), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), GSSG-reductase (yeast), glutamate, L-α-aminobutyrate, puromycin dihydrochloride, deoxycholic acid, L-γ-glutamyl-*p*-nitroanilide, glycylglycine, and *o*-phthalaldehyde were purchased from the Sigma Chemical Co., St. Louis, MO. Sodium selenite was purchased from the J. T. Baker Chemical Co., Phillipsburg, NJ. Other reagents were purchased from the Fisher Scientific Co., Chicago, IL.

Puromycin dihydrochloride (15 mg/kg) was dissolved in saline and injected intraperitoneally [19]. The animals were killed at the intervals indicated in the appropriate experiments, and the livers were perfused *in situ* with saline until fully blanched. The

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livers were removed immediately and homogenized in 1 vol. of Tris–HCl buffer (0.01 M, pH 7.5) containing 0.25 M sucrose; the kidneys were homogenized in 5 vol. of the same buffer. The 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 105,000 *g* supernatant fractions were used for the determination of GSH and GSSG and the activities of GSSG-reductase, GSH-peroxidase, and γ-glutamylcysteine synthetase. The cellular homogenates were used for the measurement of γ-glutamyl transpeptidase activity. The concentration of GSH in the whole cellular homogenates was also determined.

Glutathione concentrations were measured by a modification of the method of Cohn and Lyle [20]. The GSH was extracted using 10 vol. of the extraction mixture (1/1/1, by vol., 0.01 N HCl–5% trichloroacetic acid (TCA)–1 mM EDTA). Protein was removed by centrifugation at 5000 *g* for 10 min. To a 50 μl sample of the supernatant fraction, 1.0 ml of 0.5 M Na_2HPO_4 and 100 μl of *o*-phthalaldehyde in methanol (1 mg/ml) were added. The GSH values were detected fluorometrically using an Aminco–Bowman spectrofluorometer. The excitation wavelength was 328 nm, and the emission wavelength was 430 nm.

The GSSG levels were estimated enzymatically by a modification of the methods described by Tietze [21] and Srivastava and Beutler [22]. One-half milliliter of 0.1 M NEM was added to a 1.0 ml sample of the cytosol fraction. The mixtures were allowed to stand at 4° for 30 min and were mixed several times. The protein was precipitated by the addition of 1.0 ml of cold 10% TCA in 0.02 N HCl followed by centrifugation (5000 *g* for 10 min). The removal of the unreacted NEM was accomplished by extracting the supernatant fraction three times with 3 vol. of ether. A 100 μl sample of the supernatant fraction was used in the GSSG assay. The reaction mixture (1.0 ml) contained DTNB (0.6 mM), EDTA (3.0 mM), NADPH (0.1 μM), GSSG-reductase (1.0 E.U.), and potassium phosphate buffer (0.15 M, pH 7.6). The reaction was initiated by the addition of GSSG, and absorption at 412 nm was measured at 25°. The blank did not contain GSSG. Glutathione disulfide prepared in 0.01 N HCl was used as the standard.

The GSH-peroxidase assay was conducted by a modified coupling method of Paglia and Valentine [23] as described by Lawrence *et al.* [24]. The assay medium (1.0 ml) contained enzyme source (80–100 μg cytosol protein), GSSG-reductase (1.0 E.U.), sodium azide (NaN_3, 1.0 mM), EDTA (3.0 mM), NADPH (0.1 mM), and potassium phosphate buffer (0.1 M, pH 7.0). The reaction was initiated by the addition of H_2O_2 (0.12 mM), and the reaction rate was measured at 25°. The blank did not contain H_2O_2. One unit of enzyme activity (E.U.) was defined as 1 nmole NADPH oxidized per milligram protein per minute. The GSSG-reductase activity was assayed by the method of Massey and Williams [25]. The assay medium (1.0 ml) contained enzyme source (80–100 μg protein), EDTA (3.0 mM), bovine serum albumin (2.0 mg), NADPH (0.1 mM), GSSG (3.0 mM), and potassium phosphate buffer (50 mM, pH 7.6). The reaction was initiated by the addition of GSSG, and the rate of activity was measured at 25°. The blank did not contain GSSG. One unit of enzyme activity was defined as 1 nmole NADPH oxidized per milligram of protein per minute.

The activity of γ-glutamylcysteine synthetase was measured as described by Sekura and Meister [26]. 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 (10 mM, pH 8.2). The reaction was started by the addition of the enzyme source (1–2 mg protein). L-α-Aminobutyrate was not added to the blank. The duration of incubation was 30 min at 37°. The reaction was terminated by the addition of 1 ml TCA (10%), and the protein was precipitated by centrifuging at 5000 *g* for 10 min. The liberated phosphate (P_i) in the supernatant fraction was determined colorimetrically (720 nm) by the method of Taussky and Shorr [27]. The enzyme activity (E.U.) was defined as 1 nmole P_i released per milligram protein per minute.

The activity of γ-glutamyl transpeptidase was measured by a modification of the method described by Tate and Meister [10] for the kidney. The cellular homogenate was incubated with deoxycholic acid (1.0%) for 15 min at 25°. The assay system (1.0 ml) consisted of enzyme source (1.2–1.5 mg protein), glycylglycine (20 mM), L-γ-glutamyl-*p*-nitroanilide (2.5 mM), NaCl (7.5 mM), and Tris–HCl buffer (0.05 M, pH 8.0). The reaction was initiated by the addition of L-γ-glutamyl-*p*-nitroanilide. The rate of release of *p*-nitroaniline was followed by the increase in absorption at 405 nm. An extinction coefficient of 9.9 $mM^{-1}cm^{-1}$ was used. The enzyme activity (E.U.) was defined as 1 nmole *p*-nitroaniline formed per milligram protein per minute.

Proteins were measured by the method of Lowry *et al.* [28], with bovine serum albumin as the standard. All experiments were repeated four to six times using one rat per experiment, and the data were analyzed using Student's *t*-test. The results are presented as means ± S.D.

RESULTS

Effects of sodium selenite on cellular GSH and GSSG levels and the activities of the enzymes of GSH metabolism. Figure 1 shows the effects of treatment (s.c.) of rats with 5, 10 and 20 μmoles/kg sodium selenite (Se) on the activities of γ-glutamylcysteine synthetase and GSSG-reductase, and the level of GSH in the liver. As shown, 24 hr after administration of 10 and 20 μmoles Se/kg to rats, significant dose-related increases in the activities of the enzymes and the level of GSH were observed. The treatment of animals with 5 μmoles Se/kg did not elicit significant alterations in the activities of the synthetase and reductase or the GSH concentration. The concentration of GSH in the liver homogenates also increased in response to treatment of rats with Se (10 and 20 μmoles/kg), and the magnitude of the increases were comparable to those observed in the

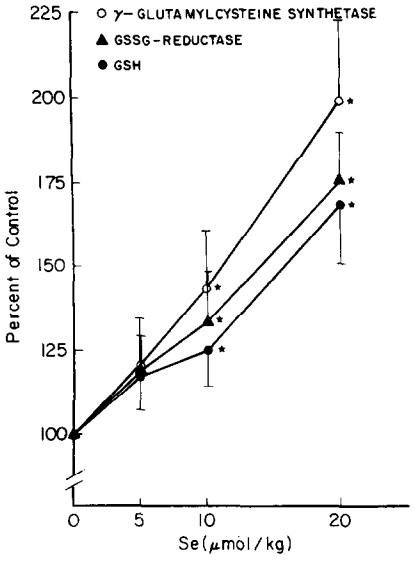

Fig. 1. Dose-related effects of sodium selenite on the cellular levels of GSH and the activities of γ-glutamylcysteine synthetase and GSSG-reductase in the rat liver. Groups of six Sprague–Dawley male rats (200–230 g) were used for each treatment. Rats were injected with sodium selenite (Na_2SeO_3, 5, 10 and 20 μmoles/kg, s.c.) and were killed 24 hr later; control animals received saline. The liver cytosol fractions were prepared and used for the measurement of the indicated variables. The control values were: GSH, 2.86 ± 0.11 mM; γ-glutamylcysteine synthetase, 25.0 ± 2.0 nmoles P_i produced·(mg protein)$^{-1}$·min^{-1}; and GSSG-reductase, 84 ± 4 nmoles NADPH oxidized·(mg protein)$^{-1}$·min^{-1}. The concentration of GSH and the activities of γ-glutamylcysteine synthetase and GSSG-reductase were determined as described in Materials and Methods. An asterisk indicates that the probability is $P \leq 0.05$.

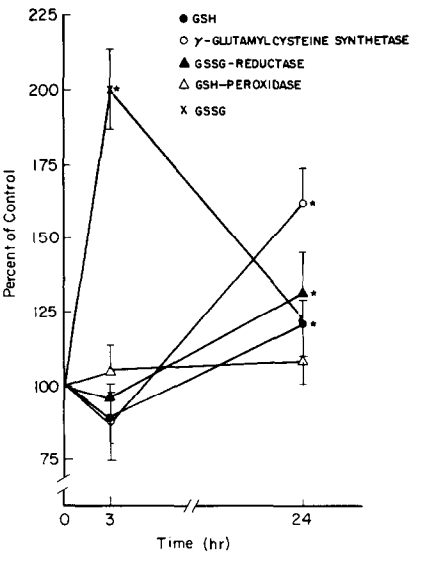

Fig. 2. Time-course of sodium selenite effects on the cellular levels of GSH and GSSG and the activities of γ-glutamylcysteine synthetase, GSSG-reductase and GSH-peroxidase in the liver. Groups of six rats were used for each treatment. The animals were killed at the indicated intervals after treatment with sodium selenite (Na_2SeO_3, 10 μmoles/kg, s.c.). The liver cytosol fractions were prepared, and the above measurements were carried out utilizing the procedures detailed in Materials and Methods. The control values were: GSH, 2.53 ± 0.21 mM; GSSG, 15.8 ± 1.0 μM; GSH-peroxidase activity, 187 ± 28 nmoles NADPH oxidized·(mg protein)$^{-1}$·min^{-1}; GSSG-reductase activity, 91 ± 9 nmoles NADPH oxidized·(mg protein)$^{-1}$·min^{-1}; and γ-glutamylcysteine synthetase activity, 22.9 ± 0.9 nmoles P_i produced·(mg protein)$^{-1}$·min^{-1}. An asterisk indicates that the probability is $P \leq 0.05$.

liver cytosol fractions (data not shown). As noted, the activity of γ-glutamylcysteine synthetase in the liver was somewhat more responsive to the administration of Se than other measured variables.

In contrast to the synthetase, the activity of γ-glutamyl transpeptidase was refractory to Se treatment (Table 1). Table 1 also presents findings on the cellular contents of GSSG and protein and the activity of GSH-peroxidase in response to various doses of Se. As noted, 24 hr after the treatment of rats with Se (5, 10 and 20 μmoles/kg) the concentration of GSSG was increased significantly; however, the magnitudes of the increases were moderate and they were not dose-related. This finding suggests that 5 μmoles Se/kg is perhaps the maximally effective dose for increasing the GSSG level. The treatment of rats with Se did not alter the peroxidase activity or the cellular protein content.

Table 1. Dose-related effects of sodium selenite on the cellular contents of GSSG and protein, and the activities of GSH-peroxidase and γ-glutamyl transpeptidase in the rat liver*

Treatment (μmoles/kg)	GSSG (μM)	GSH-peroxidase (E.U.†)	γ-Glutamyl transpeptidase (E.U.‡)	Cytosol protein (mg/g liver)
0	17.2 ± 0.8	175 ± 28	1.60 ± 0.25	58.8 ± 1.0
5	21.7 ± 0.9§	152 ± 19	2.05 ± 0.39	62.4 ± 4.4
10	20.5 ± 1.3§	187 ± 35	1.28 ± 0.37	58.2 ± 1.2
20	20.5 ± 1.1§	187 ± 16	1.79 ± 0.40	60.0 ± 2.1

* The liver cytosol of rats treated with sodium selenite, as described in the legend of Fig. 1, was used for the above measurements. The cellular levels of GSSG and the activities of GSH-peroxidase and γ-glutamyl transpeptidase were measured as described in Materials and Methods.

† E.U. is defined as 1 nmole NADPH oxidized·(mg protein)$^{-1}$·min^{-1}.

‡ E.U. is defined as 1 nmole *p*-nitroaniline formed·(mg protein)$^{-1}$·min^{-1}.

§ $P \leq 0.05$.

The time-courses of the effects of treatment of rats with Se (10 μmoles/kg) on the various parameters of GSH metabolism are shown in Fig. 2. This figure also shows the effect of Se treatment on GSH-peroxidase activity. As shown, the Se-mediated modulations of the contents of GSH and GSSG, and the activities of GSH biosynthetic enzymes in the liver were time-dependent. When rats were treated with 10 μmoles Se/kg, the level of GSH in the liver was only slightly decreased after 3 hr. At this time, however, Se treatment elicited an increase in the cellular level of GSSG. This increase was observed in the absence of a diminished activity of GSSG-reductase or a significant increase in the activity of the peroxidase. When measured at 24 hr, the GSSG level as well as the activities of the synthetase and the reductase and the concentration of GSH were increased significantly. The activity of the peroxidase 3 hr after Se treatment did not differ significantly from that observed at 24 hr after administration of the element. The time-course of the effects of treatment of rats with the large dose of Se (20 μmoles/kg) on GSH metabolism in the liver was also studied (data not shown); the pattern of the responses of the measured variables closely resembled those observed with 10 μmoles Se/kg (Fig. 2). In further studies, the cellular levels of GSH and the activities of the synthetase and the reductase 2 hr after treatment of rats with 10 and 20 μmoles Se/kg were measured. At this interval, significant decreases in the cellular levels of GSH in the treated rats, compared to the control animals, were observed; in the rats treated with 0, 10 and 20 μmoles Se/kg, the concentrations of GSH in the cytosol were 2.84 ± 0.49 mM, 2.04 ± 0.22 mM, and 1.24 ± 0.18 mM respectively. At this interval the activities of the enzymes of GSH biosynthesis remained unaltered (data not shown).

The data presented in Table 2 suggest that Se-mediated stimulation of the activities of γ-glutamylcysteine synthetase and GSSG-reductase involves the *de novo* synthesis of the enzyme proteins. Treatment of rats with the protein synthesis inhibitor, puromycin, prevented the Se-mediated increases in the activities of these enzymes. Puromycin treatment alone did not affect the activities of the reductase of the synthetase. Due to the limited duration of Se treatment (20 μmoles/kg, 11 hr), the magnitudes of the increases elicited by Se in the activities of the enzymes were not as pronounced as those observed in response to 24-hr treatment (Fig. 1). The duration of the experiment was not prolonged due to the required regimen of puromycin treatment and the toxicity of the compound.

Effects of repeated treatment of rats with sodium selenite on cellular GSH and GSSG levels, and the activities of GSSG-reductase and γ-glutamylcysteine synthetase in the liver. The effects of repeated treatment of rats with Se on the metabolism of GSH in the liver were investigated. Rats were injected three times at 24-hr intervals with 7.0 μmoles/kg of Se and were killed 24 hr after the last injection. Table 3 shows findings on the effects of this regimen of Se treatment on the levels of GSH and GSSG, and the activities of GSSG-reductase and γ-glutamylcysteine synthetase in the liver. As shown, the Se-mediated increases in the activities of the synthetase and the reductase were comparable to those produced in 24 hr by a single dose of the element (Fig. 1, 20 μmoles/kg). These findings are consistent with the above described dose-related responses of the synthetase and the reductase to Se and suggest the additive effects of low doses of Se on the activities of the enzymes of GSH biosynthesis. Similarly, the cellular level of GSH did not vary significantly from that observed in response to treatment with a single dose of 20 μmoles Se/kg. However, the concentration of GSSG was significantly higher than that observed with a single injection of the element (Fig. 1).

In vitro *studies.* The *in vitro* effects of Se on the levels of GSH and GSSG and the activities of GSSG-reductase, γ-glutamylcysteine synthetase, and γ-glutamyl transpeptidase were investigated. The addition of various concentrations of Se (10^{-7} –10^{-3} M) to the liver cytosol fractions, used as the enzyme source for GSSG-reductase, or to purified

Table 2. Inhibition by puromycin of sodium selenite-mediated increases in the activities of γ-glutamylcysteine synthetase and GSSG-reductase in the rat liver*

Treatment	GSSG-reductase (E.U.†)	γ-Glutamylcysteine synthetase (E.U.‡)
Control	88 ± 5	24.0 ± 1.8
Se	107 ± 3§	32.5 ± 2.0§
Se + puromycin	90 ± 6	24.8 ± 0.9
Puromycin	89 ± 4	24.1 ± 2.0

* Two groups of eight Sprague–Dawley male rats (200–230 g) were treated with Na_2SeO_3 (20 μmoles/kg, s.c.) or saline. One-half of each group was injected intraperitoneally with puromycin (15 mg/kg) at −1, 1, 3, 5, 7 and 9 hr after Se or saline treatments. The other rats received saline injections at the corresponding times. Animals were killed 2 hr after the last injection. The liver cytosol fractions were prepared, and the indicated enzyme activities were measured as described in Materials and Methods.

† E.U. is defined as 1 nmole NADPH oxidized · (mg protein)$^{-1}$ · min^{-1}.

‡ E.U. is defined as 1 nmole P_i produced · (mg protein)$^{-1}$ · min^{-1}.

§ $P \leq 0.05$, compared to the control animals.

Table 3. Effects of repeated sodium selenite treatment on the activities of γ-glutamylcysteine synthetase and GSSG-reductase, and the contents of GSH and GSSG in the rat liver*

Treatment	GSH (mM)	GSSG (μM)	GSSG-reductase (E.U.†)	γ-Glutamylcysteine synthetase (E.U.‡)	Cytosol protein (mg/g liver)
Control	2.60 ± 0.25	14.6 ± 3.8	100 ± 4	23.9 ± 2.1	62.4 ± 3.8
Se	3.56 ± 0.21§	27.7 ± 5.3§	143 ± 14§	35.9 ± 3.3§	65.8 ± 4.8

* Groups of four rats (200–230 g) were used. One group was treated with Na_2SeO_3 (7.0 μmoles/kg, s.c.) once every 24 hr for 3 days. The control group received saline. Twenty-four hours after the last injection the animals were killed, and the liver cytosol fractions were prepared as described in Materials and Methods. The GSH and GSSG levels and the activities of the GSSG-reductase and γ-glutamylcysteine synthetase were measured as detailed in Materials and Methods.

† E.U. is defined as 1 nmole NADPH oxidized · (mg protein)$^{-1}$ · min^{-1}.

‡ E.U. is defined as 1 nmole P_i produced · (mg protein)$^{-1}$ · min^{-1}.

§ $P \leq 0.05$, compared to the control animals.

GSSG-reductase (yeast) did not increase the reductase activity (data not shown). Similarly, when Se in the same range of concentrations was added to the γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase assay systems, no significant effect was seen (data not shown). These findings suggest that the Se-mediated increases in the activities of the synthetase and the reductase (Figs. 1 and 2) do not reflect a direct mode of action of Se.

The *in vitro* effects of Se on GSH and GSSG levels were also studied. As shown in Fig. 3, increasing concentrations of Se added to the liver cytosol preparations caused marked decreases in the levels of GSH, and increases in the GSSG values were observed. This relationship persisted up to Se concentrations of 0.4 mM; higher concentrations of the element did not further change the ratio of GSH to GSSG. These findings indicate the ability of Se to convert GSH to GSSG. The data further suggest that the reciprocal relationship observed between GSH and GSSG concentrations 2 and 3 hr after Se treatment (Fig. 2 and text) may reflect the occurrence of a similar phenomenon *in vivo*.

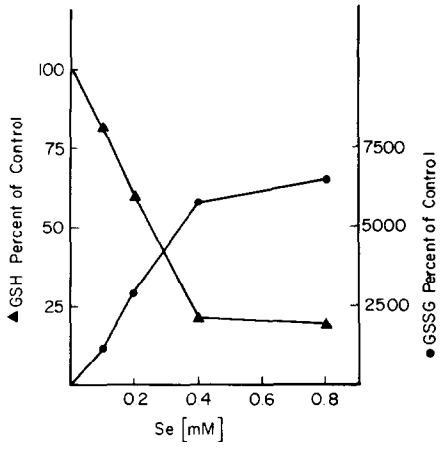

Fig. 3. *In vitro* effects of sodium selenite on GSH and GSSG levels in rat liver cytosol. The cytosol fraction was prepared from the perfused livers of male Sprague–Dawley rats as described in Materials and Methods. Sodium selenite was dissolved in 0.9% NaCl and 1–10 μl was added to 1-ml fractions of cytosol to obtain the final concentrations indicated above. The mixtures were maintained for 10 min at 0° and then the GSH and GSSG levels were measured as detailed in Materials and Methods. The control value for GSH was adjusted to 2.60 ± 0.15 mM and that of GSSG was adjusted to 15 ± 1.0 μM. Each point is the average value of three determinations and the extent of variations among the determinations was 5–10 per cent.

DISCUSSION

The experiments presented in this report described the novel properties of Se to increase the activities of γ-glutamylcysteine synthetase, the first and rate-limiting enzyme in GSH biosynthesis, and GSSG-reductase, which catalyzes the reduction of GSSG to GSH. The increases in the activities of the enzymes were accompanied by elevated cellular levels of GSH and GSSG. It is likely that the increases in GSH and GSSG levels seen 24 hr after Se (10 and 20 μmoles/kg) administration, and subsequent to repeated exposure to low doses of Se (7.0 μmoles/kg, three times), involve Se-mediated elevations of the activities of the synthetase and the reductase (Figs. 1 and 2 and Tables 2 and 3). This is supported by the finding that the activity of γ-glutamyl transpeptidase, which catalyzes the initial step in the degradation of GSH [9, 10], was unresponsive to Se treatment. This, of course, does not exclude the possibility that Se inhibited the activity of other GSH catabolic enzymes, such as cysteinylglycine-dipeptidase [29, 30]. The occurrence of increased GSH level concomitant with an elevated GSSG-reductase activity may reflect the increased activity of the synthetase. Conversely, there is a possibility that the accelerated reduction of GSSG renders NADPH the limiting factor in the reduction and/or Se causes depletion of the cofactor.

The precise molecular mechanism by which Se increases the activities of γ-glutamylcysteine synthetase and GSSG-reductase has not been established. However, according to the *in vitro* studies, the increased activities of the synthetase and the reductase clearly do not reflect activation of preformed enzymes. Rather, the results of the experiments with puromycin (Table 3) and the observation that the increased activities of GSSG-reductase and γ-glutamylcysteine synthetase were evoked in a time- and dose-dependent manner suggest that Se initiated cellular events leading to altered synthesis of the

enzyme proteins. Selenium action may involve an increased production of the reductase and the synthetase (Table 2), although the possibility of Se-mediated decreased catabolism of the enzymes cannot be ruled out. The inductive events might reflect the occurrence of a host of cellular processes such as the direct action of Se at the cellular regulatory sites for the turnover of the enzymes, an indirect response to Se-mediated disruptions of various cellular activities involved in GSH metabolism, or perturbations in the levels of GSSG to GSH. The latter possibility constitutes a plausible mechanism by which Se mediated its stimulating effects on the production of the synthetase and the reductase and is consistent with the data presented in this report.

An increase in GSSG-reductase activity may occur in response to a Se-mediated increase in GSSG levels (Fig. 2, 3 hr). Similarly, an increase in γ-glutamylcysteine synthetase activity may occur in response to the initial (2 hr after Se treatment), depletion of cellular GSH by Se. Richman and Meister [31] have suggested a feedback regulatory action of GSH on the production of the synthetase. These investigators have shown that, at physiological concentrations, GSH inhibits the formation of γ-glutamylcysteine synthetase. Although in the present study a marked decrease in GSH levels was observed only in response to the 2-hr treatment with 20 μmoles Se/kg, it is plausible that the modest decrease in GSH concentrations caused by the lower dose of Se (10 μmoles/kg) was of sufficient magnitude to trigger the cellular events leading to an increased activity of the synthetase.

The initial depletion of GSH and the elevation of GSSG seen 2–3 hr after Se administration could, in part, result from direct interaction of Se with GSH. Ganther and Hsieh [32] and Ganther [33] have demonstrated that Se can enzymatically or nonenzymatically convert GSSG to GSH, which is consistent with the observations shown in Fig. 3. According to Ganther and Hsieh [32], this reaction would represent the following mechanism:

$$4GSH + SeO_3^{2-} \longrightarrow GSSeSG + GSSG$$

It should be noted that the concentration of GSH in the cytosol fraction used for these studies was 100- to 150-fold greater than that of GSSG; therefore, the conversion of a rather small amount of GSH to GSSG would produce the observed pronounced increases in the GSSG values. This factor is most likely responsible for the observations reported in Fig. 2; as noted 3 hr after Se treatment, the marked increase in the cellular GSSG concentration was accompanied by a modest decrease in the GSH level. Moreover, it is known that perturbations in the ratio of GSSG/GSH in the cell provokes altered protein synthesis activities [34]. Accordingly, the Se-mediated alteration in the ratio of GSSG/GSH (Fig. 2) could cause modulations in the turnover rates of the synthetase and the reductase. However, Se-mediated alterations in cellular metabolic activities, such as an impaired capacity to regenerate NADPH, increased activities of GSH-utilizing enzymes, decreased GSH production subsequent to Se interaction with cysteine or other sulfur-containing intermediates in the formation of cysteine from the cystathionine pathway [35], may also trigger increased enzyme protein synthesis.

It is apparent that a host of factors, some of which are discussed above, could be involved in the Se-mediated increases in the activities of γ-glutamylcysteine synthetase and GSSG-reductase and in the cellular levels of GSH and GSSG. It is also possible that these effects of Se may be involved in the known protective effect of this element against intoxication with mercurial compounds [36–39] and cellular lipid peroxidative events [40–45]. Even though Se injection has no effect on the activity of GSH-peroxidase in these experiments, the increased GSH levels by Se may well be the mechanism by which Se protects against hepatotoxicity of free radical intermediates generated during the biotransformation of various cellular toxins.

Acknowledgements—This study was supported by Grant PHS ES 02180. We are thankful to Ms. May Szeto and Mr. Daniel McGregor for their able technical assistance.

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