PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **180**, 348–352 (1985)

Effect of Dietary Selenium Concentration and Duration of Selenium Feeding on Hepatic Glutathione Concentrations in Rats[1] (42187)

ROBERT A. LEBOEUF, KATHY L. ZENTNER, AND WILLIAM G. HOEKSTRA

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706

Abstract. Studies were conducted in rats to determine the effect of dietary selenium (Se) concentration on hepatic glutathione concentrations and enzyme activities associated with the maintenance of the cellular glutathione status. Male rats were fed 0.1, 3.0, or 6.0 ppm Se as Na_2SeO_3 for 2, 4, or 6 weeks at which time they were killed and analyses were performed. Both 3.0 and 6.0 ppm Se caused a significant dose-dependent increase in hepatic-reduced glutathione (GSH) by 4 weeks of feeding compared to 0.1 ppm Se. The increase in GSH was preceded by significant, dose-dependent increases in oxidized glutathione (GSSG) as well as the GSSG to GSH ratio. Increases in GSSG and the GSSG to GSH ratio as well as in glutathione reductase and glucose-6-phosphate dehydrogenase activities were observed by 2 weeks of high Se feeding. The current findings substantiate previous results demonstrating effects of high Se on hepatic glutathione concentrations (R. A. LeBoeuf and W. G. Hoekstra, J. Nutr. **113**:845–854, 1983) and further suggest that increased cellular GSSG concentrations or the GSSG to GSH ratio caused by 3.0 and 6.0 ppm dietary Se signals for "adaptive" changes in hepatic glutathione metabolism.
© 1985 Society for Experimental Biology and Medicine.

Selenium (Se) is an essential nutrient, required for the enzymatic activity of glutathione peroxidase (1). In the rat, the nutritional requirement for Se is currently set at 0.1 ppm (2). When fed at concentrations of approximately 4 ppm or greater, Se toxicity may result depending on other factors including diet and the age of the animal. In addition, Se concentrations moderately below those shown to be toxic in experimental animals show anticarcinogenic effects in a number of cancer producing systems. The biochemical effects of high Se are therefore of interest and are the focus of this report.

Se as sodium selenite undergoes reductive metabolism from a +4 to a −2 oxidation state utilizing reduced glutathione (GSH) and NADPH as the source of reducing equivalents (3). Catalytic oxidation of GSH by selenite can also occur (4, 5). Since alterations in cellular glutathione concentrations can have numerous effects on cell processes (6), the effect of dietary Se concentration on hepatic glutathione metabolism was studied.

A recent study from our laboratory demonstrated that 6.0 ppm Se as Na_2SeO_3 fed to rats for 6 weeks caused a significant increase in hepatic nonprotein sulfhydryls (NPSH) and oxidized glutathione (GSSG) concentrations as well as the GSSG to NPSH ratio compared to 0.1 ppm Se controls (7). While GSH was not determined on all samples, random sampling of livers from all treatment groups indicated that NPSH was approximately 94% GSH. In addition, glutathione reductase (GSSG-Rd) and glucose-6-phosphate(G-6-P) dehydrogenase activities, which are involved in the reduction of GSSG to GSH, were also significantly increased with high Se feeding. In a subsequent study conducted to determine the time course of change in glutathione concentrations upon Se exposure, a single ip acute injection of Na_2SeO_3 into rats also caused a significant increase in hepatic NPSH. The increase in NPSH was preceded by a significant increase in GSSG and the GSSG to NPSH ratio. It was proposed that increased GSSG or the GSSG to NPSH ratio signals for the "adaptive" increase in NPSH (GSH) in an attempt to maintain a nearly normal GSSG to NPSH (GSH) ratio. The "adaptive" increases in GSSG-Rd and G-6-P dehydrogenase observed with high Se feeding support this hypothesis.

Our previous study (7) examined the effect of 6.0 ppm Se on glutathione metabolism at

[1] Research supported by the College of Agricultural and Life Sciences, University of Wisconsin–Madison.

0037-9727/85 $1.50
Copyright © 1985 by the Society for Experimental Biology and Medicine.
All rights reserved.

only one time point compared to 0.1 ppm Se. Therefore, the objectives of the experiment reported here were to determine whether an intermediate concentration of 3.0 ppm Se would elicit similar effects on hepatic glutathione concentrations as was seen with 6.0 ppm Se, to determine the time course of change in hepatic glutathione concentrations upon high Se feeding, and to verify that the observed increase in NPSH with high Se feeding is due to GSH.

Methods and Materials. Male weanling rats of the Sprague–Dawley strain (Holtzman, Madison, Wis.) were housed individually in hanging wire mesh cages. They were maintained on a 12-hr light–dark cycle and had free access to food and water. The basal diet contained 15% vitamin-free casein (Teklad, Madison, Wis.), 10% corn oil, 46.3% corn starch, 23.1% glucose monohydrate, 5% salts mix, 0.5% vitamin mix, 0.1% choline chloride, and 100 IU/kg diet vitamin E as all rac-α-tocopherol as described previously (7). Se as Na_2SeO_3 was supplemented to the basal diet at 0.1, 3.0, or 6.0 ppm as a Se:glucose monohydrate premix prepared immediately prior to diet formulation. The 0.1 ppm Se supplemented diet (control) contained 0.13 ppm Se as determined fluorometrically (8) and satisfied all nutrient requirements for the growing rat as indicated by the NRC (2). All rats were first fed a diet supplemented with 0.1 ppm Se for 3 weeks and then switched to diets containing either 0.1, 3.0, or 6.0 ppm Se. Rats were fed the experimental diets for 2, 4, or 6 weeks at which time eight rats per experimental group per time point were killed and biochemical analyses were performed. All biochemical methods used were described in detail previously (7). GSH and GSSG were determined according to the method of Tietze (9) as modified by Griffith for GSSG (10). G-6-P dehydrogenase (D-glucose-6-phosphate $NADP^+$ oxidoreductase, E.C. 1.1.1.49) activity was determined according to Takata and Watanabe (11). GSSG reductase (E.C. 1.6.4.2) activity was measured according to Massey and Williams (12) and protein was determined according to Lowry *et al.* (13). Effects of Se on the variables determined were analyzed for statistical significance by Duncan's New Multiple Range test (14).

Results. Six ppm Se caused a significant reduction in body weight gain by 6 weeks of high Se feeding compared to 0.1 ppm Se while 3.0 ppm Se was without significant effect. The weight gain values for the 6-week period of Se feeding were 240 ± 6, 225 ± 5, and 217 ± 4 g for 0.1, 3.0, and 6.0 ppm Se, respectively.

Three and 6.0 ppm Se caused a significant ($P < 0.01$), dose-dependent increase in hepatic GSH concentrations compared to 0.1 ppm Se. This increase was observed by 4 weeks of Se feeding (Fig. 1) and GSH remained elevated with 3.0 and 6.0 ppm Se feeding through the remainder of the study. Preceding the increase in GSH was a significant ($P < 0.01$) increase

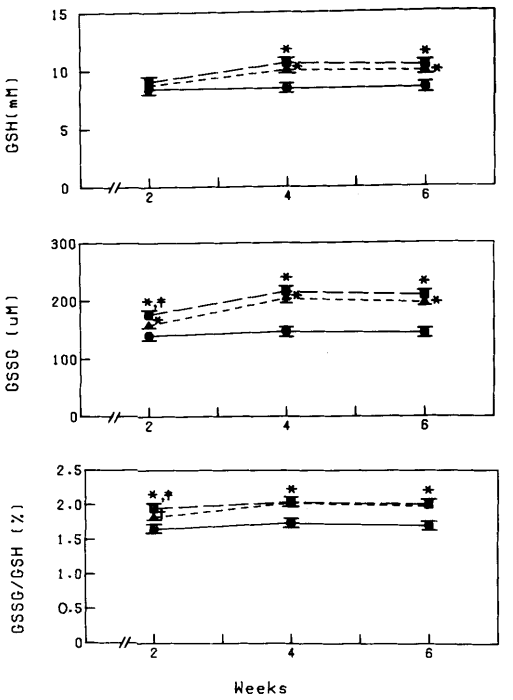

FIG. 1. Effect of dietary selenium (Se) concentration and duration of Se feeding on hepatic glutathione concentrations. Rats were fed 0.1, 3.0, or 6.0 ppm Se as Na_2SeO_3 for 2, 4, or 6 weeks at which time glutathione analysis was performed as described under Methods and Materials. Reduced glutathione (GSH), oxidized glutathione (GSSG). ●, 0.1 ppm Se; ▲, 3.0 ppm Se; ■, 6.0 ppm Se. † and * indicate values that are significantly different ($P < 0.05$ and $P < 0.01$, respectively) than 0.1 ppm Se values as indicated by Duncan's New Multiple Range test. ‡ indicates value is significantly different ($P < 0.05$) than 3.0 ppm Se value.

in hepatic GSSG concentration by 3.0 and 6.0 ppm Se compared to 0.1 ppm Se in a dose-dependent manner. Increased GSSG was observable by 2 weeks of high Se feeding (Fig. 1). The ratio of GSSG to GSH was also significantly elevated ($P < 0.05$ and $P < 0.01$ for 3.0 and 6.0 ppm Se, respectively) by 2 weeks of high Se feeding compared to 0.1 ppm Se and, similar to GSSG, remained elevated throughout the remainder of the experiment (Fig. 1). Hepatic GSH concentrations were 8.47 ± 0.08, 9.88 ± 0.11, and 10.38 ± 0.14 mM for 0.1, 3.0, and 6.0 ppm Se, respectively, at the 6-week time point. GSSG values were 144 ± 5, 196 ± 8, and 209 ± 9 μM for 0.1, 3.0, and 6.0 ppm Se, respectively, at 6 weeks of Se feeding.

GSSG-Rd and G-6-P dehydrogenase activities were significantly ($P < 0.01$) increased by approximately 40 and 74%, respectively, by both 3.0 and 6.0 ppm Se compared to 0.1 ppm Se by 2 weeks of high Se feeding (Fig. 2). G-6-P dehydrogenase and GSSG-Rd activities expressed as EU/mg protein × 10^2 were 2.35 ± 0.05 and 3.42 ± 0.03, 3.90 ± 0.05 and 4.78 ± 0.04, and 4.01 ± 0.06 and 4.80 ± 0.04 for 0.1, 3.0, and 6.0 ppm Se, respectively, at 6 weeks of Se feeding.

Discussion. The present study substantiates our previous results that 6.0 ppm Se as Na_2SeO_3 fed for 6 weeks increased hepatic GSH concentrations. The present study further demonstrates that the increase in GSH occurs by 4 weeks of high Se feeding and also occurs with 3.0 ppm dietary Se. In addition, increased hepatic GSH preceded by increased GSSG observed with high Se feeding is a kinetic pattern of glutathione change similar to that observed previously by us (7) and others (15) following a single subacute injection of Na_2SeO_3. "Adaptive" increases in hepatic GSH may represent an attempt to maintain a nearly normal ratio of GSSG to GSH. The increased GSSG-Rd and G-6-P dehydrogenase activities observed with high Se feeding would also function to maintain the ratio of GSSG to GSH in the cell by increasing the capacity to reduce GSSG. The finding that these enzyme activities were increased maximally by 2 weeks of Se feeding suggests that these enzymes represent a first line of adaptive change which occur in order to minimize the increase in cellular GSSG. Adaptive increases in GSH may indicate that the capacity of GSSG-Rd, available NADPH, or other mechanisms involved in GSSG control, such as hepatic efflux of GSSG (16, 17), have been exceeded. Changes in the protein:glutathione mixed disulfides of the cell caused by Se treatment should also be considered in future experiments due to the fact that protein:glutathione mixed disulfides contribute substantially to the total cellular glutathione pool (18, 19). Subsequent studies in our laboratory have also demonstrated "adaptive" changes in glutathione metabolism in hepatoma cells *in vitro* upon Se exposure.[2] In addition, a variety of other chemical and physical agents including metals (20), carcinogens and tumor promoters (21–23), synthetic antioxidants (24), hyperbaric oxygen (25), and hyperthermia (26) cause increased cellular GSH which in some cases may represent "adaptive" increases as is proposed for Se.

A number of processes and enzyme activities are known to be affected by an altered cellular glutathione status (6), including inhibition of protein synthesis by elevated GSSG, even in the presence of normal GSH

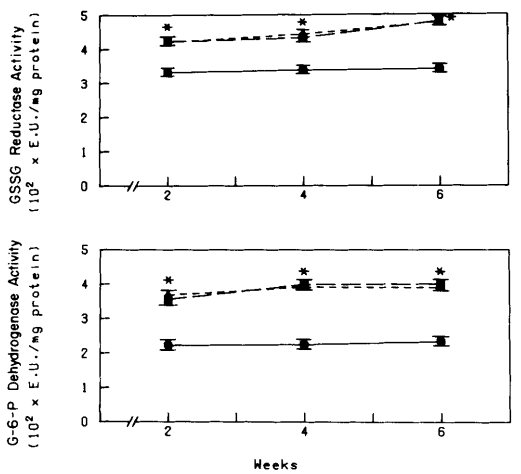

FIG. 2. Effect of dietary selenium (Se) concentration and duration of Se feeding on hepatic glutathione reductase (GSSG-Rd) and glucose-6-phosphate (G-6-P) dehydrogenase activity. See Fig. 1 for legend.

[2] Manuscript submitted for publication.

concentrations (27, 28). Inhibition of protein synthesis by elevated GSSG in reticulocyte lysates is mediated through a protein kinase which phosphorylates eucaryotic initiation factor 2 (eIF-2) and thereby decreases its efficiency of initiation of protein synthesis (29). Selenite can also inactivate eIF-2 and the effect of selenite is similarly mediated through eIF-2 phosphorylation (30). It is therefore likely that increased GSSG caused by high Se is relevant to Se inhibition of protein synthesis (31–33). This does not exclude, however, other effects of elevated GSSG or Se on cellular processes.

As discussed elsewhere[2] (7), changes in glutathione metabolism by Se may be involved in Se toxicity and in Se's anticarcinogenic effects. Three ppm Se shown in the present study to alter glutathione concentrations are well within the range of those Se concentrations shown to have anticarcinogenic effects by others.

The noteworthy findings of this study were that dietary Se concentrations of 30 and 60 times the nutritional requirement caused an overall shift in hepatic glutathione concentrations toward a more oxidized state. These changes were observed by 2 weeks of high Se feeding and preceded increases in hepatic GSH. Increased GSSG or the GSSG to GSH ratio caused by high Se feeding may initiate "adaptive" changes in hepatic glutathione metabolism.

1. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science (Washington, DC) **179**:588–590, 1973.
2. National Research Council. Nutrient requirements of the laboratory rat. In Nutrient Requirements of Laboratory Animals, Washington DC, National Academy of Sciences, pp7–37, 1978.
3. Ganther HE. Metabolism of hydrogen selenide and methylated selenides. Adv Nutr Res **2**:107–128, 1979.
4. Voegtlin C, Johnson JM, Rosenthal SM. The oxidation catalysis of crystalline glutathione with particular reference to copper. J Biol Chem **93**:435–453, 1931.
5. Tsen CC, Tappel AL. Catalytic oxidation of glutathione and other sulfhydryl compounds by selenite. J Biol Chem **233**:1230–1232, 1958.
6. Kosower NS, Kosower EM. The glutathione status of cells. Int Rev Cytol **54**:109–159, 1978.
7. LeBoeuf RA, Hoekstra WG. Adaptive changes in hepatic glutathione metabolism in response to excess selenium in rats. J Nutr **113**:845–854, 1983.
8. Oh SH, Ganther HE, Hoekstra WG. Selenium as a component of glutathione peroxidase isolated from ovine erythrocytes. Biochemistry **13**:1825–1829, 1974.
9. Tietze F. Enzymatic method for the quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem **27**:502–522, 1969.
10. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem **106**:207–212, 1980.
11. Takata K, Watanabe A. Interconvertible microheterogeneity of glucose-6-phosphate dehydrogenase in rat liver. Biochim Biophys Acta **235**:19–26, 1971.
12. Massey V, Williams CH. On the reaction mechanism of yeast glutathione reductase. J Biol Chem **240**:4470–4480, 1965.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
14. Steel RGD, Torrie JH. Principles and Procedures of Statistics. New York, McGraw–Hill, 1960.
15. Chung AS, Maines MD. Effect of selenium on glutathione metabolism: Induction of γ-glutamylcysteine synthetase and glutathione reductase in the rat liver. Biochem Pharmacol **30**:3217–3223, 1981.
16. Sies H, Gerstenecker C. Oxidation in the NADP system and release of GSSG from hemoglobin-free perfused rat liver during peroxidatic oxidation of glutathione by hydroperoxides. FEBS Lett **27**:171–175, 1972.
17. Sies H, Summer KH. Hydroperoxide-metabolizing systems in rat liver. Eur J Biochem **57**:503–512, 1975.
18. Modig H. Cellular mixed disulphides between thiols and proteins, and their possible implication for radiation protection. Biochem Pharmacol **17**:177–186, 1968.
19. Harrap KR, Jackson RC, Riches PG, Smith CA, Hill BT. The occurrence of protein-bound mixed disulfides in rat tissues. Biochim Biophys Acta **310**:104–110, 1973.
20. Sasame HA, Boyd MR. Paradoxical effects of cobaltous chloride and salts of other divalent metals on tissue levels of reduced glutathione and microsomal mixed function oxidase components. J Pharmacol Exp Ther **205**:718–724, 1978.
21. Neish WJP, Davies HM, Reeve PM. Carcinogenic azo dyes, dye binding and liver glutathione. Biochem Pharmacol **13**:1291–1303, 1964.
22. Fiala S, Mohindru A, Kettering WG, Fiala AE, Morris HP. Glutathione and gamma glutamyl transpeptidase in rat liver during chemical carcinogenesis. JNCI **57**: 591–598, 1976.
23. Kaplowitz N, Kuhlenkamp J, Goldstein L, Reeve J. Effect of salicylates and phenobarbital on hepatic glu-

tathione in the rat. J Pharmacol Exp Ther **212**:240–245, 1980.
24. Batzinger RP, Ou SL, Bueding E. Antimutagenic effects of 2(3)-tert-butyl-4-hydroxyanisole and of antimicrobial agents. Cancer Res **38**:4478–4485, 1978.
25. Kimball RE, Reddy K, Peirce TH, Schwartz LW, Mustafa MG, Cross CE. Oxygen toxicity: Augmentation of antioxidant defense mechanisms in rat lung. Amer J Physiol **230**:1425–1431, 1976.
26. Mitchell JB, Russo A, Kinsella TJ, Glatstein E. Glutathione elevation during thermotolerance induction and thermosensitization by glutathione depletion. Cancer Res **43**:987–991, 1983.
27. Kosower NS, Vanderhoff GA, Kosower EM. Inhibition of protein synthesis by glutathione disulfide in the presence of glutathione. Biochem Biophys Res Commun **45**:816–821, 1971.
28. Kosower NS, Vanderhoff GA, Kosower EM. Glutathione VIII. The effects of glutathione disulfide on initiation of protein synthesis. Biochim Biophys Acta **272**:623–637, 1972.
29. Ernst V, Levin DH, London IM. Inhibition of protein synthesis initiation by oxidized glutathione: Activation of a protein kinase that phosphorylates the α subunit of eukaryotic initiation factor 2. Proc Natl Acad Sci USA **75**:4110–4114, 1978.
30. Safer B, Jagus B, Crouch D. Indirect inactivation of eukaryotic initiation factor 2 in reticulocyte lysate by selenite. J Biol Chem **255**:6913–6917, 1980.
31. Gruenwedel DW, Cruikshank MK. The influence of sodium selenite on the viability and intracellular synthetic activity (DNA, RNA, and protein synthesis) of HeLa S3 cells. Toxicol Appl Pharmacol **50**:1–7, 1979.
32. Lewko WM, McConnell WP. Biphasic influence of selenium on cell growth and the synthesis of collagen in cultured mammary tumor cells. Fed Proc **41**:623, 1982.
33. Vernie LN, Collard JG, Eker APM, DeWildt A, Wilders IT. Studies on the inhibition of protein synthesis by selenodiglutathione. Biochem J **180**:213–218, 1979.

Received December 19, 1983. P.S.E.B.M. 1985, Vol. 180.
Accepted July 2, 1985.