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Alleviation of Silver Toxicity by Selenite in the Rat in Relation to Tissue Glutathione Peroxidase[1,2] (38697)

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Antagonism between silver (Ag) and selenium (Se) was suggested by the induction of Se deficiency signs through administration of Ag acetate in the drinking water of rats and chicks fed low-protein, vitamin E-deficient diets (1, 2). The apparent effects of this Ag-induced Se deficiency, such as the liver necrosis induced in rats, could be prevented by vitamin E, selenite, sulfur-containing amino acids, or antioxidants. These are agents which also alleviate such deficiency signs when induced by a low-Se diet. We have confirmed (3) that Ag accelerates the production of liver necrosis in rats fed a vitamin E-deficient, 8.3% casein diet similar to that used previously (1). However, we observed a 100% incidence of liver necrosis, without Ag administration, which was caused presumably by the lower Se content of the casein used in our study. In subsequent studies (3) and in those to be described, we fed a higher amount of casein (20%), in order to provide a dietary Se level which prevented the spontaneous development of liver necrosis, but hopefully would allow the Ag antagonism of Se to be manifested. Following the discoveries in this laboratory that glutathione peroxidase (glutathione H_2O_2 oxidoreductase, EC 1.11.1.9) is a Se-containing enzyme (4, 5) and that its activity is decreased in the erythrocytes, liver, and other tissues of rats fed a Se-deficient Torula yeast-based diet (6–8), we postulated (3) that Ag might be exerting its antagonistic effects on Se through an effect on the activity or biosynthesis of glutathione peroxidase (GSH-Px). Therefore, experiments were conducted to assess the effect of dietary Se intake on Ag toxicity as evaluated by growth rate, survival, and tissue Ag concentration and to study the effects of Ag and Se administration to rats on GSH-Px activity of liver, erythrocytes, kidney, and brain.

Materials and Methods. Two separate experiments were conducted with 21-day-old (55–65 g) rats of the Holtzman strain housed in individual galvanized hanging wire mesh cages. Diets and distilled water were provided *ad lib.* The average Se content of the casein-based diet, patterned after that used by Diplock *et al.* (1), was 0.02 ppm. After 1 wk of adjustment to the experimental diets the rats were assigned to treatment groups of similar mean body weight and administration of Ag, as Ag acetate[3] in the drinking water, was begun. The Ag concentration of the drinking water and rat tissues was determined by atomic absorption spectrometry. GSH-Px activity was analyzed according to the procedure developed by Mills (9) as modified previously (7). Statistical analysis was by Duncan's multiple range test (10).

In Experiment 1, designed primarily to test the effect of selenite on Ag toxicity as evidenced by any Ag-induced liver necrosis and decreased growth, 30 rats were fed the vitamin E-deficient basal diet and 30 were fed this diet supplemented with 0.5 ppm Se, supplied as Na selenite. The composition of the basal diet was as follows

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[2] A preliminary report of these experiments was presented at the meetings of the Federation of American Societies for Experimental Biology: Swanson, A. B., Wagner, P. A., Ganther, H. E., and Hoekstra, W. G. (1974). Antagonistic effects of silver and tri-*o*-cresyl phosphate on selenium and glutathione peroxidase in rat liver and erythrocytes. Fed. Proc. **33**, 693 (Abstract).

[3] J. T. Baker Chemical Co., Phillipsburg, NJ.

(in %): casein ("vitamin test"),[4] 20; glucose monohydrate (Cerelose),[5] 66.88; salt mix,[6] 4.82; vitamin mix,[7] 0.25; choline chloride, 0.0522; stripped lard,[8] 6; and cod liver oil,[9] 2. Ag was provided in the water at levels of 0, 76, and 751 ppm for a period of 52 days. At the termination of the experiment, liver and erythrocytes were analyzed for GSH-Px activity and Ag concentrations of liver and kidney were determined.

The major objective of Experiment 2 was to assess the effect of relatively long-term (15 week) administration of Ag on the GSH-Px activity of tissues from rats fed the 20% casein diet supplemented with vitamin E at 100 IU/kg (*all-rac*-alpha-tocopherol)[10] and Se (0.5 ppm as Na selenite). The diet was otherwise identical to the one used for Experiment 1, except that the cod liver oil was replaced with Cerelose. Rats were provided either 0 or 751 ppm Ag in the drinking water for 15 wk after a 1-week period of adjustment to the diet. Liver, kidney, brain, and erythrocytes were analyzed for GSH-Px activity.

Results and Discussion. Growth rate and survival of rats on Experiment 1 are shown in Fig. 1. Ag at 751 ppm in the water supply severely depressed ($P < 0.05$) growth of rats fed the low-Se (0.02 ppm), vitamin E-deficient basal diet as early as 14 days and resulted in death of four of ten rats by 39–46 days. There was no gross evidence of liver necrosis, but histological observations were not conducted. Dietary Se (0.5 ppm) dramatically improved growth and survival of rats receiving the higher (751 ppm) level of Ag and completely overcame the growth depression caused by 76 ppm Ag.

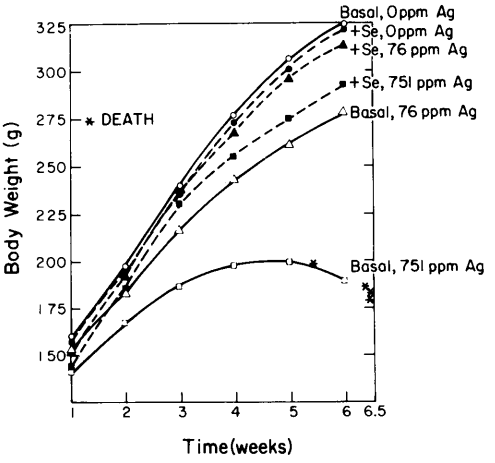

FIG. 1. Effect of Ag in the drinking water on the growth and survival of rats fed low-Se (0.02 ppm) or Se-supplemented (0.5 ppm) diets deficient in vitamin E (Experiment 1). Ag administration was begun after one week of adjustment to the diets. Deaths (*) occurred only in the low-Se group receiving 751 ppm Ag. Means are from ten rats per group.

The protective effect of Se against Ag toxicity in the absence of vitamin E was associated with an increase, rather than a decrease, in tissue Ag (Fig. 2). Dietary Se produced a three-fold increase in the Ag concentration of liver from rats given 751 ppm Ag and approximately a twofold increase in rats given 76 ppm Ag. The apparent increase in kidney Ag concentration caused by Se at both levels of Ag was not statistically significant ($P > 0.05$). For a given level of Ag in the drinking water, the Ag concentration of liver always exceeded that of kidney (< 0.05).

Liver and erythrocyte GSH-Px activity (Table I) of rats fed the low-Se (0.02 ppm) basal diet lacking in vitamin E was comparable to that previously reported for rats fed a Se-deficient Torula yeast-based diet containing 0.008 ppm Se (7). After rats were fed the Se-deficient diet for 59 days,

[4] Nutritional Biochemicals, Cleveland, OH.

[5] Cerelose from CPC International, Englewood Cliffs, NJ.

[6] The salt mix provided the following in gm/kg of diet: $NaH_2PO_4 \cdot H_2O$, 23; $CaCO_3$, 18.2; KCl, 3.5; Na_2CO_3, 1.2; $MgSO_4$, 1.95; ferric citrate, 0.15; $MnSO_4 \cdot H_2O$, 0.15; $ZnSO_4 \cdot 7H_2O$, 0.06; NaF, 0.00025; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.002; $CoSO_4 \cdot 7H_2O$, 0.01; $CuSO_4$, 0.018; KI, 0.0003.

[7] The vitamin mix provided the following in mg/kg of diet: thiamin HCl, 10; riboflavin, 19; niacin, 90; pyridoxine HCl, 10.94; calcium pantothenate, 90; folic acid, 2; 0.1% trituration of cyanocobalamin, 30; inositol, 90; *p*-aminobenzoic acid, 90; menadione, 0.146; vitamin D_3 (500,000 U.S.P. units/g), 3.2; vitamin A (500,000 IU/g), 6; cerelose (glucose monohydrate), 2058.6.

[8] Eastman Kodak, Rochester, NY.

[9] Rexall Drug, St. Louis, MO.

[10] See Footnote 4.

their liver GSH-Px was undetectable and erythrocyte GSH-Px was about 25% of that observed in rats supplemented with 0.5 ppm Se. Ag at 76 and 751 ppm in the water reduced ($P < 0.05$) liver GSH-Px of rats fed 0.5 ppm Se to 30% and 4%, respectively, of the activity of the 0 ppm Ag control group. In this experiment, no effect ($P > 0.05$) of Ag on the GSH-Px activity of the erythrocytes was detected. However, an effect of Ag on erythrocyte GSH-Px was subsequently observed in experiments with rats fed vitamin E-adequate diets (Fig. 3 and other data not shown).

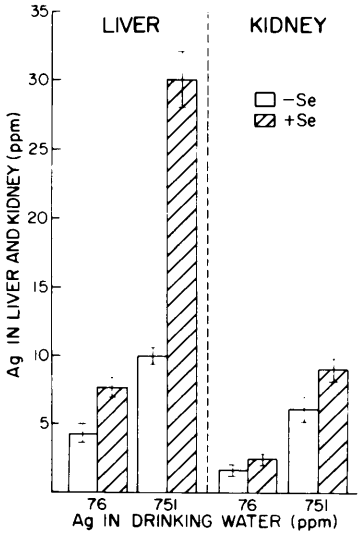

FIG. 2. Effect of dietary Se on the Ag concentration of liver and kidney after the administration of two levels of Ag in the drinking water for a period of 52 days (Experiment 1). Means are from 3 to 5 rats per group; error bars represent SEM.

In Experiment 2 (Fig. 3) the administration of 751 ppm Ag in the water for a period of 15 wk to rats fed a casein-based diet adequate in Se (0.5 ppm) and vitamin E (100 IU/kg) significantly ($P < 0.05$) decreased GSH-Px activity in liver, erythrocyte, and kidney to 5%, 37%, and 38% of the 0 ppm Ag control, respectively. The apparent decrease of brain GSH-Px due to Ag to 78% of control was not significant ($P > 0.05$). GSH-Px activities in the tissues of these Se-supplemented rats given Ag for 15 wk are roughly comparable to those previously observed in tissues of rats fed a Se-deficient Torula yeast-based diet for 13 wk (8). Rats in Experiment 2 survived the entire period of Ag administration and maintained their body weights at about 85% that of the controls.

Our studies confirm the ameliorative effect of selenite against Ag toxicity and demonstrate a profound reduction by Ag in the activity of the selenoenzyme, GSH-Px, in the liver. Whether such decreases in GSH-Px represent enzyme inhibition by

TABLE I. EFFECT OF Ag ON GLUTATHIONE PEROXIDASE ACTIVITY OF RAT LIVER AND ERYTHROCYTES (Experiment 1).

Diet	Ag in drinking water[a] (ppm)	Glutathione peroxidase activity: Erythrocytes (EU/mg Hemoglobin)	Erythrocytes (% of Control)	Liver (EU/10 mg Fresh liver)	Liver (% of Control)
+Se[b]	0	30[c] ± 0.5[d]	100	108[c] ± 2[d]	100
+Se	76	30 ± 0.3	100	33 ± 0.4	30
+Se	751	31 ± 1	103	4 ± 0.03	4
Basal[e]	0	8.3 ± 0.1	100	N.D.[f]	
Basal	76	8.3 ± 0.1	100	N.D.	
Basal	751	8.2 ± 0.1	99	N.D.	

[a] Ag, as Ag acetate, was administered in the drinking water to the rats for 52 days following 1 wk of adjustment to the diets.

[b] 0.5 ppm Se as Na selenite was added to the 20% casein basal diet.

[c] Means of three to five animals per group.

[d] Values are presented as means ± SEM.

[e] The Se content of the 20% casein basal diet was 0.02 ppm.

[f] GSH-Px activity was not detectable in the livers of rats fed the low-Se (0.02 ppm) basal diet.

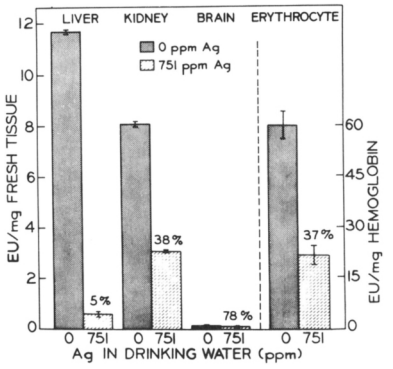

FIG. 3. Effect of Ag in the drinking water on GSH-Px activity of liver, kidney, brain, and erythrocytes from rats fed a Se-supplemented (0.5 ppm) diet adequate in vitamin E (100 IU/kg). Ag administration was begun after one week of adjustment to the diet and continued for 15 wk. Means are from three rats per group; error bars represent SEM.

Ag or decreased biosynthesis of the enzyme due to interference with Se metabolism is unresolved. We did not observe gross signs of Ag-induced liver necrosis in rats fed the low-Se (0.02 ppm), 20% casein diet lacking in vitamin E. This lack of liver necrosis contrasts with results obtained earlier with the 8.3% casein diet (1, 3) and is probably related to the adequate intake of sulfur-containing amino acids from the 20% casein diet, but not from the 8.3% casein diet. Because cysteine is a precursor of reduced (GSH) glutathione, the sulfur-containing amino acids may determine the amount of GSH available for both the nonenzymic and the GSH-Px catalyzed decomposition of peroxides. Even if GSH-Px is depleted, as it was in the liver of the rats fed the low-Se basal diet (Table I), the GSH dependent (9), nonenzymic destruction of peroxides alone could presumably prevent the development of liver necrosis. The proposed roles of vitamin E, Se, sulfur-containing amino acids, and GSH-Px in the prevention of liver necrosis are described elsewhere in more detail (4, 7, 8, 11).

The biochemical mechanisms involved in the modification of chronic Ag toxicity by selenite are poorly understood and open to speculation. The observed growth depression and mortality may be attributed in part or entirely to effects of Ag other than an induced deficiency of liver GSH-Px, as this selenoenzyme was undetectable in rats fed either the low Se diet alone or the same diet with Ag in their drinking water. However, GSH-Px activity of kidney or other tissues could be important in protecting the rat against the toxic effects of Ag. Possibly other biologically active forms of Se may be antagonized by Ag to an even greater extent than is GSH-Px. It seems unlikely that Se decreases Ag toxicity by decreasing tissue Ag, since our results show that Ag is increased in liver and possibly in kidney. A diversion of Ag by Se from critical targets to non-toxic binding sites and of Se by Ag to biologically inactive forms within the cell could explain the increased tissue concentration of Ag, the concomitant modification of the Ag toxicity, and the induction of Se-deficiency defects.

Summary. Dietary Se (0.5 ppm Se supplied as sodium selenite to a casein-based diet containing 0.02 ppm Se and lacking in vitamin E) prevented the growth depression observed in rats receiving 76 ppm Ag in the water supply and markedly improved growth and survival of those given 751 ppm Ag. The Ag concentration of liver and possibly of kidney was increased by Se. Liver glutathione peroxidase activities from rats fed 0.5 ppm Se and given 76 and 751 ppm Ag for 52 days in their water were, respectively, 30% and 4% of those from control rats fed 0.5 ppm Se without Ag. In rats fed a diet, adequate in vitamin E (100 IU/kg) and Se (0.5 ppm as sodium selenite), administration of 751 ppm Ag in the water for 15 wk reduced liver GSH-Px activity to 5% of that from control rats receiving no Ag. GSH-Px activity of erythrocytes and kidney was decreased by Ag to 37% and 38%, respectively, of control values. It is concluded that *in vivo* administration of Ag dramatically decreased liver GSH-Px in rats fed Se-supplemented diets with or without vitamin E. Furthermore, supplemental Se (0.5 ppm) prevented the growth depression and mortality caused by Ag in rats fed a diet lacking vitamin E, while increasing the Ag concentration of liver and kidney.

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