PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 209–215 (1982)

Stimulation of Lipid Peroxidation *in Vivo* by Injected Selenite and Lack of Stimulation by Selenate[1] (41333)

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Abstract. Male weanling rats were raised on diets based on torula yeast which were deficient in vitamin E and selenium, or supplemented with these substances. They were injected intraperitoneally with sodium selenite at 2 mg Se/kg or with sodium sulfite to give the same level of sodium. Following injection, lipid peroxidation *in vivo* was estimated by monitoring the production of ethane, a volatile product formed on the peroxidation of ω-3-unsaturated fatty acids. In the hour following injection, vitamin E- and selenium-deficient rats injected with selenite produced 15 times as much ethane as did controls injected with sulfite. All rats in this group died from 1 to 4 hr after injection. Rats fed diets supplemented with selenite showed only a two- to threefold stimulation of ethane production by selenite and ¾ survived. Rats fed diets supplemented with vitamin E did not produce more ethane in response to selenite injection and ¾ survived. All four rats supplemented with both vitamin E and selenium survived without showing increased ethane production. Thus, the increased vulnerability of vitamin E- and selenium-deficient rats to acute selenite toxicity may involve peroxidation *in vivo*. Rats fed diets supplemented with vitamin E could survive at least twice as much selenite as rats deficient in selenium and vitamin E. Seven-day survival figures for rats fed the basal diet and injected with selenite were: 1 mg Se/kg, 7/8; 2 mg Se/kg, 0/8. For rats supplemented with vitamin E the figures were 2 mg Se/kg, 7/8; 4 mg Se/kg, 2/8. When sodium selenate was injected into rats deficient in vitamin E and selenium at 3 mg Se/kg it caused acute mortality without increasing peroxidation *in vivo*.

There is much evidence that lipid peroxidation is associated with the tissue defects resulting from vitamin E and selenium deficiency in animals, and may be important in the etiology of these defects. Tissues from vitamin E- and selenium-deficient animals peroxidize *in vitro* more rapidly than tissues from animals adequate in these nutrients (1). It has recently been found by utilizing the respiratory ethane technique that vitamin E- and selenium-deficient animals show elevated levels of lipid peroxidation *in vivo* (2). Furthermore, iron and carbon tetrachloride, which accelerate lipid peroxidation *in vivo*, cause increased mortality in vitamin E- and selenium-deficient rats (3, 4).

Bunyan *et al.* (5) found that selenite stimulated lipid peroxidation *in vitro*. They showed that α-tocopherol added *in vitro* completely prevented dialuric acid-induced peroxidation and hemolysis of vitamin E-deficient erythrocytes. However, when the same level of tocopherol was added with 64 ppm sodium selenite, rapid hemolysis and peroxidation occurred.

The present experiments were designed to determine whether injected selenite or selenate promote lipid peroxidation *in vivo* in vitamin E- and selenium-deficient and adequate rats. We also checked the association of increased peroxidation with increased mortality.

Materials and Methods. Male weanling Sprague–Dawley rats were housed and fed diet as described in the preceding paper (6). Lipid peroxidation *in vivo* was estimated by measuring expired ethane. Injections, ethane collection, and ethane and carbon dioxide estimation were all done as described previously (6).

[1] Research supported by the College of Agricultural and Life Sciences, University of Wisconsin–Madison, and by United States Public Health Program Grant AM 14881.

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0037-9727/82/020209-07$01.00/0

Determination of nonprotein sulfhydryl groups was by a modification of the method of Sedlak and Lindsay (7). One gram of lung or kidney or 0.5 g of liver was homogenized in 19 ml of 0.2 *M* disodium EDTA, pH 4.6. The EDTA solution was bubbled vigorously with nitrogen for 5 min prior to use. Three 5.0-ml aliquots of homogenate were taken. To one was added 0.1 ml of a reduced glutathione standard in 0.02 *M* disodium EDTA. Five milliliters of 10% TCA solution was added to each tube and the tubes were mixed by inversion. The precipitate was centrifuged for 15 min at 1000*g*. One milliliter of supernatant was added to 2.0 ml of 0.4 *M* Tris, pH 8.9. Then 0.1 ml of 0.005 *M* Ellman's reagent in absolute methanol was added and the tubes were mixed by inversion. The absorbance at 412 nm was read within 5 min of adding Ellman's reagent. Reagent blanks and external glutathione standards were run with each batch of homogenates. The data presented have been adjusted using internal standards to compensate for losses during processing of approximately 10%. Nonprotein sulfhydryl groups were estimated using the molar extinction coefficient of 13,900 which was experimentally determined using glutathione.

All confidence intervals given in this paper are standard errors of the means.

Results. *Effects of toxic doses of selenate and selenite on the lipid peroxidation in vivo and survival of selenium- and vitamin E-deficient rats.* Rats were fed for 50 to 58 days a selenium- and vitamin E-deficient diet containing 5% cod liver oil, and for 2 days the same diet lacking cod liver oil. The rats were divided into five treatment groups: one was injected with 3 mg selenium/kg as sodium selenate; another with a dose of sodium sulfate equivalent in sodium to the sodium selenate; a third with 3 mg selenium/kg as sodium selenite; a fourth with sodium sulfite equivalent in sodium to the sodium selenite; and the fifth group was left uninjected.

Immediately after treatment, the rats were placed in ethane collection chambers (4) and survival (Table I) and ethane production (Table II) were followed. Rats injected with sodium selenite died between 1 and 3 hr after injection. At 1 hr, selenite-injected rats had produced 12 times as much ethane as had rats in any of the other groups. Three of the four rats injected with sodium selenate died. The first two of these died between 2 and 3 hr after injection. Neither at 2 hr nor at 3 hr did the selenate-injected rats display elevated ethane production.

The large rise in ethane production caused by selenite injection suggested that lipid peroxidation might be important in the toxicity of selenite in vitamin E- and selenium-deficient rats. The absence of any such rise in selenate-injected rats suggested that lipid peroxidation is not important to the acute toxicity of selenate.

In this and similar experiments, selenite and selenate have caused somewhat different patterns of gross pathological lesions. Selenite caused the appearance of bloody froth at the nose and mouth. Lungs were extensively hemorrhagic and the thoracic cavity contained large amounts of bloody fluid. The small intestines and the walls of the peritoneal cavity were stained red, and the kidneys were abnormally congested. Selenate caused the appearance of bloody fluid at the nose and mouth, and in the thoracic cavity in only one of seven rats examined. Two other rats showed straw-colored fluid at the nose and mouth, and in the thoracic cavity. Lung abnormalities were always observed from selenate ranging from congestion to definite hemorrhage. The small intestines were stained reddish-orange. Kidneys appeared normal.

TABLE I. EFFECTS OF SELENIUM AS SELENITE OR AS SELENATE ON THE SURVIVAL OF SELENIUM- AND VITAMIN E-DEFICIENT RATS[a]

Substance injected	Fraction surviving				
	1 hr	2 hr	3 hr	4 hr	24 hr
Uninjected	4/4	4/4	4/4	4/4	4/4
Sodium sulfate	4/4	4/4	4/4	4/4	4/4
Sodium sulfite	4/4	4/4	4/4	4/4	4/4
Sodium selenate	4/4	4/4	2/4	1/4	1/4
Sodium selenite	4/4	1/4	0/4	0/4	0/4

[a] 3 mg/kg selenium injected ip.

TABLE II. EFFECTS OF SELENATE, SELENITE, SULFATE, OR SULFITE ON THE ETHANE PRODUCTION OF VITAMIN E- AND SELENIUM-DEFICIENT RATS

Substance injected	Ethane production (nmole/100 g body wt)			
	1 hr	2 hr	3 hr	4 hr
Uninjected	0.6 ± 0.1*,[a]	1.3 ± 0.3*	1.8 ± 0.2*	2.2 ± 0.3*
Sodium sulfate	0.9 ± 0.4*	1.6 ± 0.4*	1.7 ± 0.2*	2.4 ± 0.2*
Sodium sulfite	0.8 ± 0.2*	1.0 ± 0.1*	1.8 ± 0.2*	1.7 ± 0.1*
Sodium selenate	0.6 ± 0.2*	1.6 ± 0.1*	1.8 ± 0.1*	1.8 ± 0.3*
Sodium selenite	12.8 ± 0.7**	15.4 ± 0.2**	—	—

[a] At each time ethane production values of the treatment groups were compared using Duncan's multiple range test. Numbers not followed by the same superscript differ significantly at $P < 0.05$, according to this test.

Effects of dietary vitamin E and selenite on the in vivo peroxidation and survival of rats injected with selenite. Rats were fed for 59 to 72 days on Torula yeast-based diets: one group was fed the basal diet; one was supplemented with 0.5 ppm selenium as sodium selenite; one was supplemented with 200 IU vitamin E/kg diet; and one was supplemented wtih 0.5 ppm selenium and 200 IU vitamin E/kg. Two days prior to ethane collection the rats were shifted to diets lacking cod liver oil. Rats were injected with 2 mg selenium/kg as sodium selenite or with sodium sulfite to give the same amount of sodium. They were placed immediately in ethane collection chambers and survival (Table III) and ethane production (Table IV) were monitored. All of the rats fed the basal diet and injected with selenite died within 4 hr. At 1 hr after injection, the last reading before deaths began to occur in this group, selenite-injected basal rats had produced 14 times as much ethane as sulfite-injected controls.

Dietary vitamin E prevented, and dietary selenite largely prevented the selenite-caused rise in ethane production. Both substances also protected against selenite-caused mortality. The data from this experiment are consistent with the hypothesis that lipid peroxidation contributes to the increased toxicity of selenite in vitamin E- and selenium-deficient rats.

TABLE III. EFFECTS OF DIETARY VITAMIN E AND SELENITE ON THE SURVIVAL OF RATS INJECTED WITH SODIUM SELENITE[a]

Diet	Fraction surviving				
	1 hr	2 hr	4 hr	1 day	7 days
Basal	4/4	2/4	0/4	0/4	0/4
+E	4/4	4/4	3/4	3/4	3/4
+Se	4/4	4/4	4/4	4/4	3/4
+E +Se	4/4	4/4	4/4	4/4	4/4

[a] 2 mg Se/kg injected. Four rats from each dietary group were injected with the same dose of sodium as sodium sulfite. All of these rats survived.

One of the rats supplemented with vitamin E died between 2 and 4 hr after selenite injection. No evidence was found to associate this death with lipid peroxidation. Both at 2 and at 4 hr after injection the ethane production by this rat was less than the average ethane production of vitamin E-supplemented, selenite-injected rats.

Magnitude of protection by vitamin E. In the ethane collection experiments mentioned earlier, vitamin E appeared to protect against acute selenite toxicity. To confirm this effect and to establish its magnitude, rats were fed for 40 days a basal Torula yeast-based diet or the diet supplemented with 200 IU/kg vitamin E. The rats were then injected with sodium selenite at doses ranging from 0.5 to 4.0 mg selenium/kg and survival was tabulated (Table V). Vitamin E-supplemented rats tolerated at least twice as much sodium selenite as did vitamin E-deficient rats.

Effect of injected selenite on rat respiration. Some fraction of the selenite-caused increase in ethane production could be caused by an overall increase in respiration. Eight rats fed for 62 to 63 days a selenium- and vitamin E-deficient diet were used to check the effect of injected selenite on respiration. The rats were shifted for 2 days to cod liver oil-free diets. Four were injected

TABLE IV. EFFECTS OF DIETARY VITAMIN E AND SELENITE ON ETHANE PRODUCTION OF RATS INJECTED WITH SELENITE OR SULFITE[a]

Diet	Substance injected	Ethane production (nmole/100 g body wt) 1 hr	2 hr	4 hr
Basal	Sulfite	1.1 ± 0.7*	1.9 ± 0.4*	3.0 ± 0.7
	Selenite	15.4 ± 0.4	17.1 ± 1.0	—
+E	Sulfite	0.8 ± 0.3	1.4 ± 0.4	1.6 ± 0.5
	Selenite	1.2 ± 0.6	1.7 ± 0.8	2.6 ± 1.0
+Se	Sulfite	0.4 ± 0.2	0.6 ± 0.1**	1.4 ± 0.1**
	Selenite	0.8 ± 0.1	1.9 ± 0.4	2.8 ± 0.5
+E +Se	Sulfite	0.2 ± 0.2	0.5 ± 0.2	1.0 ± 0.1
	Selenite	0.3 ± 0.2	0.8 ± 0.3	1.1 ± 0.2

[a] 2 mg Se/kg injected.
* Sulfite- and selenite-treated groups are significantly different. $P < 0.001$, Student's t test.
** $P < 0.05$.

with 2 mg Se/kg sodium selenite. Four were injected with the same dose of sodium as sodium sulfite. Ethane and carbon dioxide production were measured for 1 hr.

None of the rats died during this period, and none showed bloody froth at the nose or mouth. Selenite-injected rats produced 15.2 ± 0.9 nmole ethane/100 g body weight and 5.5 ± 0.3 mmole CO_2/100 g. Sulfite-injected rats produced 0.4 ± 0.1 nmole ethane/100 g and 5.3 ± 0.2 mmole CO_2/100 g. Selenite clearly caused a large increase in ethane production without significantly affecting respiration.

Effects of selenite on tissue glutathione levels. Selenite is a very effective catalyst for the oxidation of glutathione (8). If selenite greatly depleted the reduced glutathione concentration of a tissue, this would deprive glutathione peroxidase of a required substrate and might serve to stimulate peroxidation. Since lung was the tissue most obviously damaged in these studies, it was decided to check lung levels of nonprotein sulfhydryls after selenite injection.

TABLE V. EFFECT OF DIETARY VITAMIN E ON THE SURVIVAL OF SELENIUM-DEFICIENT RATS INJECTED WITH SODIUM SELENITE

Diet	Selenite dose (mg/kg)	Fraction surviving 1[a]	2	3	7
Basal	0.5	8/8	8/8	8/8	8/8
	1.0	7/8	7/8	7/8	7/8
	2.0	0/8	0/8	0/8	0/8
	4.0	0/8	0/8	0/8	0/8
+E	0.5	8/8	8/8	8/8	8/8
	1.0	8/8	8/8	8/8	8/8
	2.0	8/8	7/8	7/8	7/8
	4.0	7/8	4/8	2/8	2/8

[a] Time in days.

A preliminary experiment was done to establish more accurately the time course of ethane production in basal rats injected with 2 mg Se/kg as selenite. Acceleration of ethane production began between 15 and 30 min after injection. Accordingly, we decided to examine nonprotein sulfhydryl levels at 30 min after injection.

Eight rats were fed for 52 to 53 days a basal diet and shifted for 2 days to a diet lacking cod liver oil. Four were injected with 2 mg selenium/kg as sodium selenite, and four were left uninjected. The rats were placed in ethane collection chambers and ethane production was measured for 30 min. They were then killed by decapitation and the nonprotein sulfhydryl content of their lungs was determined using Ellman's reagent. In one-half hour the uninjected rats produced 0.2 ± 0.1 nmole of ethane/100 g body weight; selenite-injected rats produced 3.6 ± 0.3. Lung nonprotein sulfhydryl contents of uninjected rats were 2.5 ± 0.1 μmole/g while selenite-injected rats had 2.0 ± 0.2 μmole/g, a decrease which was

only marginally ($P < 0.1$) significant. It was concluded that at the time the selenite-injected rats were producing ethane rapidly, their lungs showed little or no decrease in nonprotein sulfhydryls.

In a similar experiment, nonprotein sulfhydryl levels of lung, kidney, and liver were examined 1 hr after the injection of basal rats with selenite at 2 mg selenium/kg. Selenite-injected rats produced 18.3 ± 2.9 nmole ethane/100 g while uninjected rats produced 1.3 ± 0.3. Lung showed a drop of nonprotein sulfhydryls from 2.0 ± 0.2 μmole/g tissue for the uninjected rats to 1.2 ± 0.1 for the selenite-injected rats. Liver and kidney glutathione levels of the two groups did not differ significantly. In view of the timing and magnitude of the decrease of lung nonprotein sulfhydryls, it seems unlikely that a glutathione drop in this tissue caused the large rise in peroxidation *in vivo*. Whether any of these organs is an important site of peroxidation *in vivo* is, of course, unknown.

Discussion. Dietary selenite and dietary vitamin E both reduce the mortality caused by injected selenite. Both dietary supplements also reduce the tendency of injected selenite to cause peroxidation *in vivo*. These observations are consistent with the hypothesis that lipid peroxidation may be important to the toxicity of selenite in vitamin E- and selenium-deficient animals.

The ability of vitamin E to protect against selenite toxicity seems explainable in terms of its role as a fat-soluble antioxidant (9). The mechanism by which dietary selenite protects against selenite-caused peroxidation and mortality seems less clear. Selenium is an important component of the enzyme glutathione peroxidase, which can metabolize lipid hydroperoxides and hydrogen peroxide (9). However, the protection against selenite-caused lipid peroxidation by dietary selenite may not be entirely or even largely due to high levels of the selenoenzyme glutathione peroxidase. The body has mechanisms for detoxifying selenite by converting it into methylated derivatives (10, 11). If these mechanisms are inducible, they might be more active in rats fed 0.5 ppm selenium than in rats fed a selenium-deficient diet. It is not possible to say on the basis of our data how much of the drop in lipid peroxidation is caused by glutathione peroxidase and how much by a more rapid detoxification of the injected selenite.

In vitro studies have shown selenite to be a much more potent inhibitor than selenate of several enzyme activities. Potter and Elvehjem (12) found that the succinic oxidase activity of chick kidney homogenates was very readily inhibited by selenite under conditions in which selenate caused no inhibition. Succinic dehydrogenase, the key enzyme for succinic oxidase activity, is an SH-containing enzyme. Studies on tissue slices and homogenates have shown that enzymes lacking SH groups are not susceptible to selenite inhibition, while most enzyme systems requiring the SH groups are susceptible (13). Selenite has also been shown to inhibit purified SH enzymes *in vitro* (14), and to oxidize glutathione and other low-molecular-weight SH-containing molecules (8).

Potter and Elvehjem (12) found, however, that selenate did inhibit tissue oxygen uptake significantly during 2-hr incubations, but not during shorter incubations. They suggested that selenate inhibited only after conversion to selenite. Rosenfeld and Beath (15) found that 8 ppm selenite blocked completely the motility of guinea pig intestine but 75 ppm selenate had no effect. They also demonstrated that liver, spleen, and blood contained heat-labile factors capable of reducing selenate to selenite.

It appears, therefore, that animals can convert selenate to selenite, and such conversion appears to be required for inhibition of the activities studied *in vitro*. This has led to the claim that selenate is only toxic after conversion to selenite (16).

However, the data presented here show that selenite or some metabolite of selenite causes extensive peroxidation *in vivo*. The protection offered by vitamin E against both peroxidation and mortality suggests that lipid peroxidation is important in the

selenite-caused toxicity. Yet, selenate caused mortality without significantly elevating *in vivo* peroxidation, and thus apparently without extensive conversion to selenite. This is not the only possible interpretation of the data. It is also possible that the injected selenite caused damage at two types of sites. At one site the selenite could accelerate peroxidation. At the other, little peroxidation but extensive damage of another sort such as sulfhydryl oxidation or blocking might occur. Conversion of selenate to selenite at only the second type of site could also account for the results.

Sodium selenite is administered to lambs orally or by ingestion to prevent white muscle disease, a condition caused by selenium deficiency and exacerbated by vitamin E deficiency (17, 18). Occasionally selenite administration has caused an acute fatal toxicosis characterized by damage to the lungs and kidneys (17, 19). In view of the increased toxicity of selenite in vitamin E- and selenium-deficient rats, it seemed possible that some of these fatalities might be due to an increased toxicity of selenite in vitamin E- and selenium-deficient lambs. However, a search of the literature yielded no evidence of enhanced selenite toxicity in lambs from farms previously affected by white muscle disease. Caravaggi *et al.* (20) established an LD_{50} for intramuscularly injected selenite at 0.45 mg selenium/kg using 8- to 10-week-old lambs from a farm where white muscle disease had not been reported. In another experiment by Caravaggi and Clark (21), using 2- to 4-week-old lambs reared on a property where selenium responsive diseases had been reported, 9/20 lambs died after the injection of 5 mg selenium per lamb as sodium selenite. This corresponded to doses of 0.6 to 1.0 mg selenium/kg. Field reports from veterinarians have shown that orally administered selenite can be fatal to lambs at doses of 1 to 2 mg selenium/kg (19, 22). However, a well-established value for the LD_{50} of oral selenite in vitamin E- and selenium-adequate lambs is lacking. The currently available data neither point to an enhanced toxicity of selenite in selenium- and vitamin E-deficient lambs, nor rule out the possibility that this may occur.

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Received April 1, 1981. P.S.E.B.M. 1982, Vol. 169.