Tumorigenesis, Metabolism, Speciation, Bioavailability, and Tissue Deposition of Selenium in Selenium-Enriched Ramps (*Allium tricoccum*)

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Ramps (*Allium tricoccum*) were grown either in a mixture of vermiculite and peat moss or hydroponically with various concentrations of selenium as sodium selenate. The concentrations used were from 30 to 300 mg of selenium/kg of vermiculite−peat moss or from 10 to 120 mg/L in the hydroponic solutions. Levels as high as 784 mg of selenium/kg were obtained in the ramp bulbs when grown with high levels of selenium in the vermiculite−peat moss, and up to 600 mg of selenium/kg was obtained hydroponically. The predominant form of selenium in the ramp bulbs at all concentrations of selenium was Se-methylselenocysteine, with lower amounts of selenate, Se-cystathionine, and glutamyl-Se-methylselenocysteine. There was a ∼43% reduction in chemically induced mammary tumors when rats were fed a diet with Se-enriched ramps. Dietary Se-enriched ramps for rats did not result in excessive tissue selenium accumulation or undesirable side effects. Bioavailability studies with rats indicated that selenium in ramps was 15−28% more available for regeneration of glutathione peroxidase activity than inorganic selenium as selenite. Therefore, Se-enriched ramps appear to have potential for the reduction of cancer in humans.

Keywords: *Ramps; Allium tricoccum; selenium; speciation; tumorigenesis; tissue accumulation; rats; bioavailability*

INTRODUCTION

A number of animal studies have shown that selenium will reduce chemically and virally induced tumors (Combs and Gray, 1998). This is consistent with epidemiological studies with humans suggesting an inverse relationship between selenium intake and certain cancers (Shamberger and Frost, 1968; Schrauzer et al., 1977). These data took on additional significance when it was shown that supplementation of American subjects with selenium as enriched yeast significantly reduced cancers of the prostate, colon, and lung (Clark et al., 1996).

The most common form of selenium available commercially as a human dietary supplement is selenium-enriched yeast (Clark et al., 1996), but the major selenocompound present in this product is typically selenomethionine (Semet; Kotrebai et al., 1999). One possible disadvantage of Semet administration is that selenium from this source tends to accumulate in tissues (Whanger and Butler, 1988), and thus it is desirable to find effective selenium sources that have high anticarcinogenic activity but which do not lead to tissue selenium accumulation. One possible approach is the enrichment of certain vegetables with selenium. Se-enriched onions and garlic (Ip and Lisk, 1994) or broccoli (C. Ip, unpublished data) have been shown to significantly reduce chemically induced mammary tumors in rats, but the tissue accumulation of selenium was not

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Figure 1. Picture of ramps showing the round bulbs with flat leaves.

markedly elevated. Although not as effective as Se-enriched garlic, regular garlic also appears to have some anticarcinogenic activity in this tumor model (Ip and Lisk, 1992). Se-enriched broccoli has been recently shown to possess anticarcinogenic activity using the aberrant crypts foci model (Finley et al., 2000). Enriched broccoli significantly reduced the number of crypts in colons of rats, providing further evidence of the feasibility of Se-enriched vegetables as anticarcinogenic agents.

Although some minor differences exist, the organosulfur compounds in ramps and garlic have been shown to be generally similar (Calvey et al., 1997, 1998), and thus ramps would be expected to take up selenium like garlic because plants with a high sulfur content take up more selenium than those with a low sulfur concentration. On the basis of their stronger odor, ramps appear to contain more volatile sulfur compounds than garlic. Therefore, because Se-enriched garlic was shown to markedly reduce chemically induced mammary tumors, it was speculated that Se-enriched ramps would also be effective in the reduction of these tumors in rats. This was the basis of the present studies, but in addition the speciation of selenium in enriched ramps, the accumulation of selenium in tissues, methods for enriching ramps with selenium, and the bioavailability of selenium in ramps are also reported.

MATERIALS AND METHODS

Growth of Ramps. A picture of ramps is given in Figure 1. These plants have round bulbs like wild onions but, in contrast, the leaves are flat rather than tubular. Because there was no information on growing ramps under controlled conditions, initial work was performed to determine if they would grow in a peat moss−vermiculite mixture using procedures similar to those for garlic (Ip et al., 1992). The peat moss used was Bacto Michigan Peat (Michigan Peat Co., Houston, TX; 22.73 kg/bag), and the horticultural vermiculites (Schundler Co., Metachen, NJ; 7.27 kg/bag) were purchased from Wetzels, Harrisonburg, VA. The peat moss was mixed at ratio of about 2:1 (w/w) with the vermiculite. As soon as the ramps emerged in the wild (which was usually the first or second week of April), they were transplanted to beds containing the peat moss−vermiculite mixture. The ramps were obtained from Cole Knob, located above the village of Trout (Greenbrier County), WV.

For the pilot study, ~6.4 kg of the peat moss−vermiculite mixture was placed in plastic tubs (50 cm diameters by 20 cm deep), and 35 ramp plants were placed evenly through this mixture. Planted pots were watered with tap water as needed and remained outside near Trout, WV. About 28 g of Peters Professional water soluble fertilizer (20−20−20 N−P−K) was added to each pot to maintain vegetative growth until the plants became dormant, which was near the end of May, and the ramp bulbs were harvested. Various concentrations of selenium as sodium selenate up to 300 mg/kg of mixture (based on dry weight at the beginning) were added to this growth mixture by dissolving in water and spraying on the growth mixture. This was applied 1 week after the ramps were transplanted to this bed.

Some ramps were grown hydroponically in tubs containing 53 L of water with various concentrations (up to 120 mg of Se/L) of selenium as sodium selenate plus 28 g of Peters Professional water soluble fertilizer (20−20−20 N−P−K). The soil was carefully removed from the roots, and each plant was placed in slots of a Styrofoam pad so that the roots protruded beneath this pad. The Styrofoam pads with plants were floated on top of the nutrient solution containing the selenium and fertilizer, and the plants were again grown outdoors near Trout, WV. A plastic sheet was placed over these containers during cold nights when frost was forecast. The ramp bulbs were harvested when the tops of plants died near the end of May.

To obtain enough ramps for a rat-feeding study, a large growth bed was constructed in the woods east of the dairy barns at Virginia Polytechnic Institute and State University (Virginia Tech). A wooden frame 13 m long and 1.3 m wide was lined on the sides and bottom with a 6 mL plastic sheet to contain the selenium from the underlying soil. Eighteen bags (7.27 kg/bag) of vermiculite and 12 bags (22.73 kg/bag) of peat moss were mixed in a portable feed mixer and dispersed in this bed to a depth of ~13 cm. Immature ramp plants were transplanted to this bed at a density of ~450 plants/m^2. They were watered to keep the mixture moist at all times. About 3.6 kg of 10−10−10 N−P−K fertilizer (without sulfur) was applied 1 week after transplanting, and the ramp bulbs were harvested after the plants become dormant, which was near the end of May.

The first experiment was conducted in 1996. About 20 mg of selenium as sodium selenate/kg of bed (based on original dry weight of 403 kg) was sprayed in an aqueous solution in three equal portions on this bed with the first spray 1 week after transplanting. The second and third spray treatments were applied, respectively, 1 and 2 weeks afterward. The ramp bulbs from this experiment were used for the bioavailability and selenium tissue deposition studies. The second growth experiment was conducted the following year, but 120 mg of selenium as sodium selenate/kg of potting medium was sprayed as an aqueous solution in three equal portions as described for the first experiment. The bulbs from this experiment were used for the rat tumor study.

After the ramp bulbs were harvested, they were cleaned with a pressure washer and frozen at −20 °C. These frozen bulbs were mixed with crushed dry ice, ground throughly with a bowl chopper (Hobart model VCM 20, Hobart Manufacturing, Troy, OH), and freeze-dried.

Mammary Tumor Study. The tumor study was conducted as described previously (Ip et al., 1992; Lu et al., 1996) except that methylnitrosourea (MNU) was used as the carcinogen. Ninety pathogen-free female Sprague−Dawley rats were purchased from Charles River Breeding Laboratories (Wilmington, MA) at 45 days of age. All animals were fed the AIN-76A basal diet (Reeves et al., 1993) for several days to acclimatize them to the powdered ration. Each rat was injected with MNU i.p. at a dose of 50 mg/kg of body weight at 50 days of age, and 1 day afterward they were randomized into three

groups. The first group was fed the basal diet, which contained ~0.1 mg of selenium/kg. The second group of rats was fed the basal diet containing 1.2% freeze-dried ramp powder, which was shown by analysis to contain 0.12 mg of selenium/kg. These control ramps were harvested from the wild and prepared for inclusion in the diet similarly to the Se-enriched ones. The third group of 30 rats was fed the basal diet with 1.2% Se-enriched ramps, which provided a concentration of 3 mg of selenium/kg of diet. All animals were palpitated for mammary gland tumors once per week, and the experiment was continued for 24 weeks. At necropsy, all tumors were excised and fixed for histological evaluation. Only confirmed adenocarcinomas are reported in the results. Samples of liver, kidney, muscle, mammary gland, and plasma were collected from each rat, frozen with liquid nitrogen, and sent on dry ice by air to Oregon State University for selenium analysis.

Bioavailability and Tissue Selenium Deposition Study. The bioavailability study was conducted as described previously (Butler et al., 1991). The same AIN-76A diet was used except that torula yeast (Rhinelander Paper Co., Rhinelander, WI) was substituted for the casein. Thirty-five weanling male Sprague−Dawley rats were purchased from Bantin and Kingman (Fremont, CA) and fed the Se-deficient diet for 5 weeks to deplete their selenium stores. This diet was shown by analysis to contain ~20 ng of selenium/g. They were then divided into seven groups of five animals per each group, and one group was continued on the same low-selenium diet. Selenium either as sodium selenite or as Se-enriched ramps was incorporated into the diets to provide either 0.05, 0.10, or 0.15 mg of selenium/kg. After the rats were fed these diets for 4 weeks, they were killed while under ether anesthesia by decapitation with a guillotine. Blood, muscle (leg), liver, and testes were removed for selenium analysis. Samples of blood and liver were prepared for glutathione peroxidase (GPX) assays. The blood was lysed (1:60 ratio) with distilled water for GPX assay. Liver was homogenized (1:6 ratio) in 0.1 M potassium phosphate buffer, pH 6, with a Potter Elvehjem homogenizer and centrifuged for 15 min at 12000*g* to obtain the supernatants for assay of GPX.

A total of 25 weanling male rats, again purchased from Bantin and Kingman (Fremont, CA), was used to study how selenium in ramps was deposited in various tissues. They were divided into five groups and fed the same basal torula yeast diets with additions of either 0.1 mg of selenium as sodium selenate/kg, 3 mg of selenium as either sodium selenate, selenomethionine (Semet), selenium-enriched ramps, or 1.5 mg of selenium as Semet plus 1.5 mg of selenium as enriched ramps per kilogram for 8 weeks. At this time the rats were killed as noted above, and blood, muscle (leg), liver, kidneys, and testes were removed for selenium analysis.

Speciation of Selenium in Ramps. The speciation of selenocompounds in ramp bulbs at various selenium concentrations was performed by high-performance liquid chromatography−inductively coupled plasma mass spectrometry (HPLC-ICPMS) (Bird et al., 1997a,b; Kotrebai et al., 1999). Briefly, dried, ground ramp bulbs were extracted using hot water or enzymatic extraction (Protease XIV) and chromatographed using either trifluoroacetic acid (TFA) or heptafluorobutyric acid (HFBA in methanol) as ion-pairing agents in the reversed phase HPLC mobile phase. The column was a 15 cm, 5 μm particle, Symmetry Shield RP8 Waters column, and the chromatograms were obtained using Se-82 specific element detection by interfaced ICPMS. Compounds were identified using retention times with matching standards plus spiking of the sample with the appropriate pure standard compounds against which the retention times were also matched.

Selenium Analysis and GPX Assays. After wet digestion with nitric and perchloric acids, selenium concentrations in all samples were determined according to a semiautomated method (Brown and Watkinson, 1977) using an autoanalyzer (Beilstein and Whanger, 1986). GPX was assayed by the coupled enzyme procedure using hydrogen peroxide as the substrate (Paglia and Valentine, 1967). Protein concentrations of the samples were determined with the Folin phenol reagent (Lowry et al., 1951).

Table 1. Uptake of Selenium by Ramps Grown in a Bed of Peat Moss and Vermiculite

expt	Se added to mixture (mg/kg)	content of Se in ramp bulbs[a] (mg/kg)
1	none	0.2 ± 0.02
	30	120 ± 32
	60	140 ± 7
	90	177 ± 5
	120	235 ± 19
2	120	250 ± 25
	150	405 ± 29
	180	524 ± 38
	210	517 ± 25
3	210	507 ± 28
	240	643 ± 28
	270	784 ± 63
	300	674 ± 36

[a] Values are means ± standard errors.

Table 2. Uptake of Selenium by Ramps Grown Hydroponically

expt	concn of Se (mg/L)	content of Se in bulbs[a] (mg/kg)
1	10	88 ± 19
	20	142 ± 5
	30	252 ± 10
2	30	230 ± 23
	50	325 ± 25
	70	335 ± 9
	90	432 ± 14
3	90	527 ± 36
	120	608 ± 42

[a] Values are means ± standard errors.

Statistical Analysis. The data were subjected to statistical analysis using analysis of variance, the Student−Newman−Keuls procedure, and calculation of regression equations (Steel and Torrie, 1980). Regression equations were based upon levels that included the content in the basal diet, which were 0.02, 0.07, 0.12, and 0.17 mg of selenium/kg. Bioavailability was calculated by dividing the slope for selenium in ramps by that for selenite.

RESULTS

Selenium Uptake by Ramps. Various increases of selenium in the growth mixture generally resulted in proportional increases of the selenium content of the ramp bulbs (Table 1). For the highest selenium concentrations used, 300 mg/kg, a level of 674 mg of selenium/kg was obtained in the ramp bulbs, which is slightly lower than the levels achieved with 270 mg of selenium/kg in the growth media. None of these selenium levels resulted in any inhibition of the growth of the ramps, based on the appearance of the plants and the yield of bulbs.

Similar results were obtained when ramps were grown hydroponically (Table 2). A concentration of 608 mg of selenium/kg of bulb was obtained for the highest level of selenium (120 mg/L) used. Again, in general, there was a proportional increase of selenium in the ramp bulbs with each increase of selenium in solution, and none of the concentrations of selenium used resulted in inhibition of growth, based on the appearance of the plants and the yield of the bulbs.

When 20 mg of selenium/kg of growth medium was used in the large bed, ~48 mg of selenium/kg was obtained in the ramp bulb. There was no difference in the selenium content regardless of whether they were

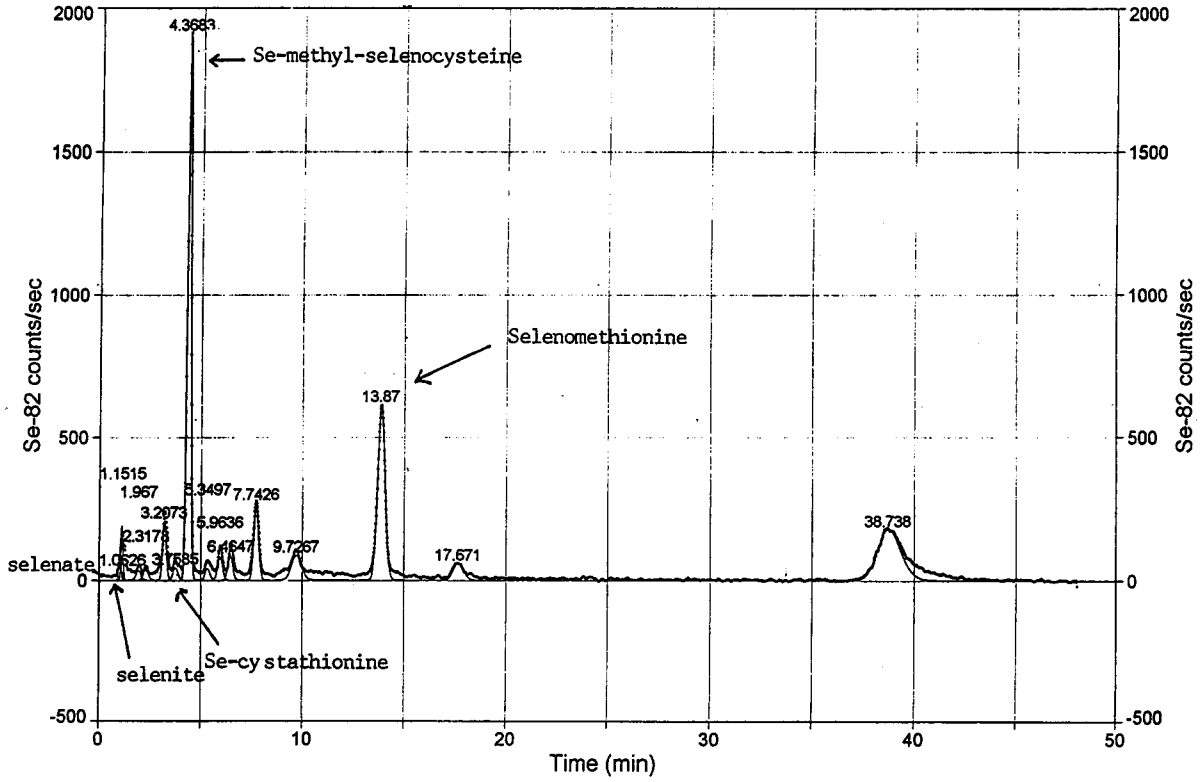

Figure 2. Chromatogram of Se-enriched ramp bulbs using HFBA as the ion-pairing agent on HPLC-ICPMS. The conditions of this chromatography are described under Materials and Methods.

freeze-dried (48 ± 7 mg) or dried (48 ± 6 mg) at 70 °C in an oven. Thus, it was concluded that volatile selenocompounds did not constitute any major amount of the total selenium content. This preparation in which 20 mg of selenium/kg was employed in the large bed was used in the bioavailability and tissue deposition studies. When 120 mg of selenium/kg of mixture was used, a concentration of 252 ± 34 mg of selenium/kg of bulbs was obtained. This is the preparation used for the tumorigenic experiment.

Speciation of Selenium in Ramps. A typical chromatogram of the ramp bulb preparation obtained with HFBA as an ion-pairing reagent is given in Figure 2; this indicates that Se-methylselenocysteine is the major selenocompound in this preparation. This chromatogram shows the elution positions of Semet, Se-cystathionine, selenite, and selenate. The HFBA additive allows considerable resolution at the beginning of the chromatogram, but late-eluting compounds are retained for an inordinate length of time. In contrast, TFA gives poor early resolution but a more acceptable overall elution time (data not shown). Hence, both additive systems are typically used in parallel when required to gain maximum information.

Table 3 gives the percentage distribution of the selenocompounds with different concentrations of selenium in the bulbs. Regardless of the selenium concentration, there appears to be a constant percentage distribution of selenocompounds in the ramp bulbs. The highest percentage of selenate was in the ramp bulbs, with 252 mg of selenium/kg, which was the preparation used for the tumor experiment. The hurricane of the spring of 1997 occurred near the first of May, and water stood in this bed for several weeks. The plants did not grow very well, and it is postulated the flooded bed caused an altered composition of selenocompounds in these particular bulbs as compared to the other ones.

Table 3. Speciation of Selenium in Se-Enriched Ramp Bulbs

Se content in ramp bulbs (μg/g)	% distribution			
	selenate	Se-cysta-thionine	Se-methyl selenocysteine	glutamyl-Se-methyl-selenocysteine
48	25	3	35	1
230	15	2	50	2
252	42	5	35	1.4
405	25	0.5	44	1.5
524	22	1.5	44	

Some volunteer bulbs not harvested the previous year emerged the following spring and were analyzed for selenium before the plants went dormant for the summer. The bulbs contained 77 ± 24 mg of selenium/kg, but surprisingly the leaves analyzed contained 255 ± 13 mg of selenium/kg. The percentage distributions of the selenocompounds in the bulbs were as follows: selenate, 1; selenite, 1.5; Se-methylselenocysteine, 33; and Semet, 20. Therefore, it appears that ramps harvested 1 year later have a distribution of selenocompounds much different from that of those grown for 2 months after being transplanted to the growth medium. Interestingly, Semet (16% of total) was the only identifiable selenocompound in the leaves from these plants, and there were three major and up to five minor unidentified selenocompounds in the leaf preparations (data not shown). Therefore, the leaves appear to have a completely different group of selenocompounds from that found in the bulbs.

Tumor Experiment. There was a ~43% reduction of mammary tumors in rats fed the Se-enriched ramps as compared to controls (Figure 3). However, the inclusion of regular ramps in the diet did not have any significant effect upon tumor incidence. Statistical differences in the incidence of tumors between the rats fed

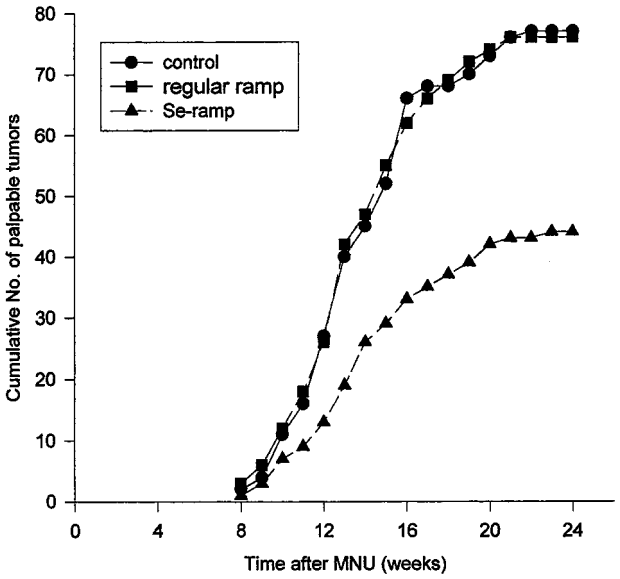

Figure 3. Cumulation of palpable tumors in rats fed control diet or this diet plus either regular ramps or Se-enriched ramps.

Table 4. Tissue Concentration of Selenium from Rats Fed a Control Diet or Control Diet with either Regular Ramps or Se-Enriched Ramps

	treatment		
tissue	controls[a]	ramps[a]	Se ramps[a]
liver	3.7 ± 0.4^{a}	3.5 ± 0.7^{a}	5.4 ± 0.7^{b}
kidney	5.1 ± 0.7^{a}	5.3 ± 0.6^{a}	8.9 ± 0.8^{b}
muscle	0.47 ± 0.08^{a}	$0.46 \pm \pm 0.05^{a}$	0.94 ± 0.09^{b}
mammary gland	0.27 ± 0.14	0.30 ± 0.10	0.36 ± 0.14
plasma	0.42 ± 0.04^{ac}	0.38 ± 0.05^{ad}	0.46 ± 0.04^{b}

[a] Concentration is expressed as μg of selenium per g of tissue (liver, kidney, muscle, and mammary gland) or per mL (plasma). Superscripts a and b within a row indicate significant difference at the 1% level ($P < 0.01$) and superscripts c and d significant differenence at the 5% level ($P < 0.05$).

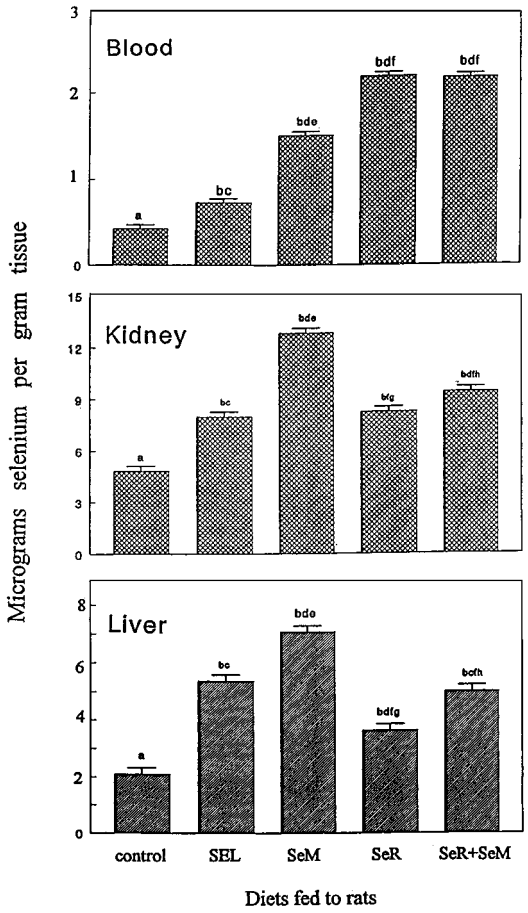

Figure 4. Accumulation of selenium in blood, kidney, and liver from rats fed the basal diet (control) or basal diet plus either 3 mg of Se/kg as either selenate (SEL), selenomethionine (SeM), Se-enriched ramps (SeR), or a mixture of selenomethionine (SeM) plus enriched ramps (SeR). The bars represent means ± standard errors, and those with different letters are significantly different ($P < 0.05$).

the Se-enriched diet and those fed the other two diets started to appear at 10 weeks and afterward following treatments with the carcinogen. Even though Figure 3 shows the cumulative number of palpable tumors with time after treatment with the carcinogen, similar trends were obtained when the number of rats with mammary tumors was plotted against time after treatment with MNU (data not shown).

Tissue Deposition of Selenium and Bioavailability. The selenium concentrations in tissues from the rats used in the tumor experiment are shown in Table 4. There were no significant differences in the selenium concentration in the mammary glands from rats among the three treatment groups, and there was no significant difference in the selenium content in liver, kidney, or muscle from rats fed regular ramps versus those fed the control diet. The selenium content in the plasma from rats fed the regular ramp diet was significantly lower than that from controls. Except for the mammary glands, the selenium concentration in all tissues examined was higher in rats given Se-enriched ramps than in these tissues from the other two groups.

Semet resulted in the greatest deposition of selenium in liver and kidney, but ramps or ramps plus Semet resulted in the highest concentration of selenium in the blood (Figure 4). There were no differences in the blood selenium content between rats fed the enriched ramps and those fed the mixture of Semet and ramps (Figure 4, top), but there was a difference in kidneys (Figure 4, middle) and liver (Figure 4, bottom). The concentration of selenium was intermediate in liver, kidneys, and blood from rats fed selenate as the selenium source in comparison to those given Semet or the control diet.

As for liver and kidneys, the highest concentration of selenium in muscle and testes was from those rats fed Semet in the diet (Figure 5). The concentrations of selenium in testes were similar in rats fed the diet with selenate, Se-enriched ramps, and the mixture of ramps plus Semet (Figure 5, bottom), but this was not true for the muscle (Figure 5, top). The selenium content in the muscle from rats given selenate was not significantly different from that in this tissue of rats fed the control diet, but both were lower than in muscle from rats fed the diets with enriched ramps or the mixture of ramps and Semet.

Selenium in ramps is more available for regeneration of GPX activity in both blood and liver than is selenium as selenite (Figure 6). By dividing the slopes of the regression lines of that for Se-enriched ramps by that for selenite, 1.28 and 1.14 ratios are obtained, respectively, for blood and liver. By this criterion, the selenium in ramps is 14 and 28% more availabile, respectively, for regeneration of GPX activity in liver and blood. Analysis of blood and liver from these rats indicated that selenium was lower in rats fed the diet with 0.05 mg of selenium as selenite but higher in these tissues of rats

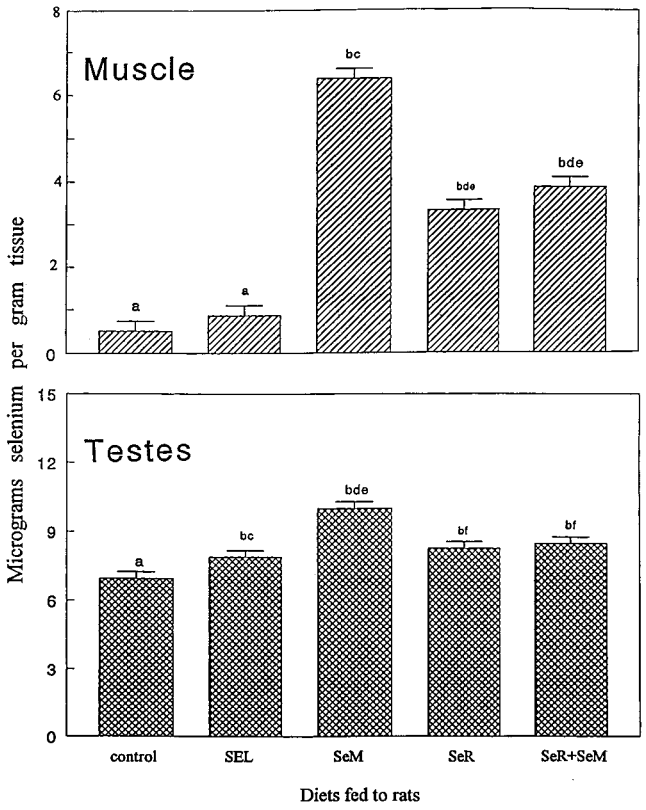

Figure 5. Accumulation of selenium in muscle and testes of rats fed the basal diet (control) or basal diet plus either 3 mg of Se/kg as either selenate (SEL), selenomethionine (SeM), Se-enriched ramps (SeR), or selenomethionine (SeM) plus enriched ramps (SeR). The bars represent means ± standard errors, and those with different letters are significantly different ($P < 0.05$).

fed the enriched ramps at 0.10 mg of selenium/kg and higher only in the blood from rats fed the enriched ramps at 0.15 mg of selenium/kg (data not shown).

DISCUSSION

Reduction of tumors with Se-enriched ramps (Figure 3) is consistent with results from Se-enriched garlic (Ip et al., 1992), onions (Ip and Lisk, 1994), and broccoli (Ip, unpublished data). However, regular garlic (Ip et al., 1992) and broccoli (Ip, unpublished data), respectively, reduced the tumor incidence by 32 and 37% as compared to controls, whereas this effect was not found with regular onions (Ip and Lisk, 1994) or ramps (Figure 3). The reasons for which the tumor incidence was reduced by regular garlic or broccoli but not by regular onions or ramps are not known. However, because the sulfur compounds in ramps are similar to those in garlic (Calvey et al., 1997, 1998), it was surprising that regular ramps did not have an effect upon tumor incidence. In the investigations of various plants, our intent was to find a selenium source with excellent anticarcinogenic activity and nutritional value that did not cause excessive tissue accumulation of selenium.

Se-methylselenocysteine as the major selenocompound in ramp bulbs (Figure 2 and Table 3) is consistent with that reported for Se-enriched garlic, onions, and broccoli (Cai et al., 1995). Se-methylselenocysteine has been shown to be one of the most effective anticarcinogenic selenocompounds examined to date (Ip and Ganther, 1993). It is intriguing that plants apparently produce this selenocompound as a defense mechanism against selenium toxicity, but this is in turn beneficial to the animal with respect to inhibition of tumors.

The preliminary evidence indicates that the chemical composition of selenocompounds may be completely different in ramps transplanted to growth beds than those grown from bulbs, on the basis of the results of plants harvested the second year. Even though Se-methylselenocysteine was still the major selenocompound present, the percentage of Semet was much higher than with transplanted plants. The much higher content of selenium in the leaves is intriguing, and further work is planned to investigate this. Likewise, except for Semet, the chemical composition of selenocompounds in the leaves appears to be completely different from the bulbs, and plans are to investigate their identity. There is apparently no information on the selenocompounds in leaves from enriched garlic or tops from enriched onions, and it will be interesting to determine if they are similar in this part from all three plants. Therefore, it may be more desirable to plant ramp bulbs in the growth medium in the fall and harvest the plants the following spring to simulate their usual growth patterns.

Similar to Se-enriched garlic and onions (Ip and Lisk, 1994), there was less accumulation of selenium from enriched ramps in tissues than from Semet (Figures 4 and 5) but less than that from selenate in liver (Figure 4, bottom) and more than that from selenate in muscle (Figure 5, top). In contrast to Se-enriched garlic and onions, the selenium contents in the kidneys from either selenate or enriched ramps were the same (Figure 4, middle), but ramps resulted in higher levels of selenium in the blood (Figure 4, top), which was even higher than from Semet. Similar effects of ramps on selenium levels in blood from rats used for the bioavailibility study were noted (data not shown). Regular ramps caused a lower plasma selenium level than controls in the rats used in the tumor study (Table 4). Therefore, chemical components in ramps appear to alter the blood selenium levels in ways that were not found with enriched garlic or onions.

The concentrations of selenium needed in the growth medium to obtain elevated uptake of selenium by ramps were much higher than reported for garlic (Ip et al., 1992). This is attributed to their much shorter growing season. The ramps grew in the Se-enriched beds for only 2 months, whereas the garlic was grown for 6 months in a bed with 20 mg of selenium/kg to achieve a concentration of 150 mg of selenium/kg of garlic bulb. Even with the highest selenium concentration used (300 mg/kg; Table 1), there was no evidence of toxicity to these plants, and thus higher levels are needed for toxic effects. Enriching the growth medium to achieve a selenium content of 1300 mg/kg in garlic bulbs resulted in plants that did not look healthy (Ip, personal observations). It cannot be determined from the present data what level of selenium will be toxic to ramps.

Although ramps with elevated selenium content show significant potential for inhibiting tumor formation in humans, ramp plants have not been shown to grow easily in culture and are usually collected from the wild. The difficulty in culturing ramps is caused by multiple factors. Ramps are perennial plants that grow in colonies in the wild along the Appalachian Mountain region from northern Georgia to Quebec, Canada. Ramps are most easily vegetatively propagated by bulbs because they have a complex multiple dormancy that

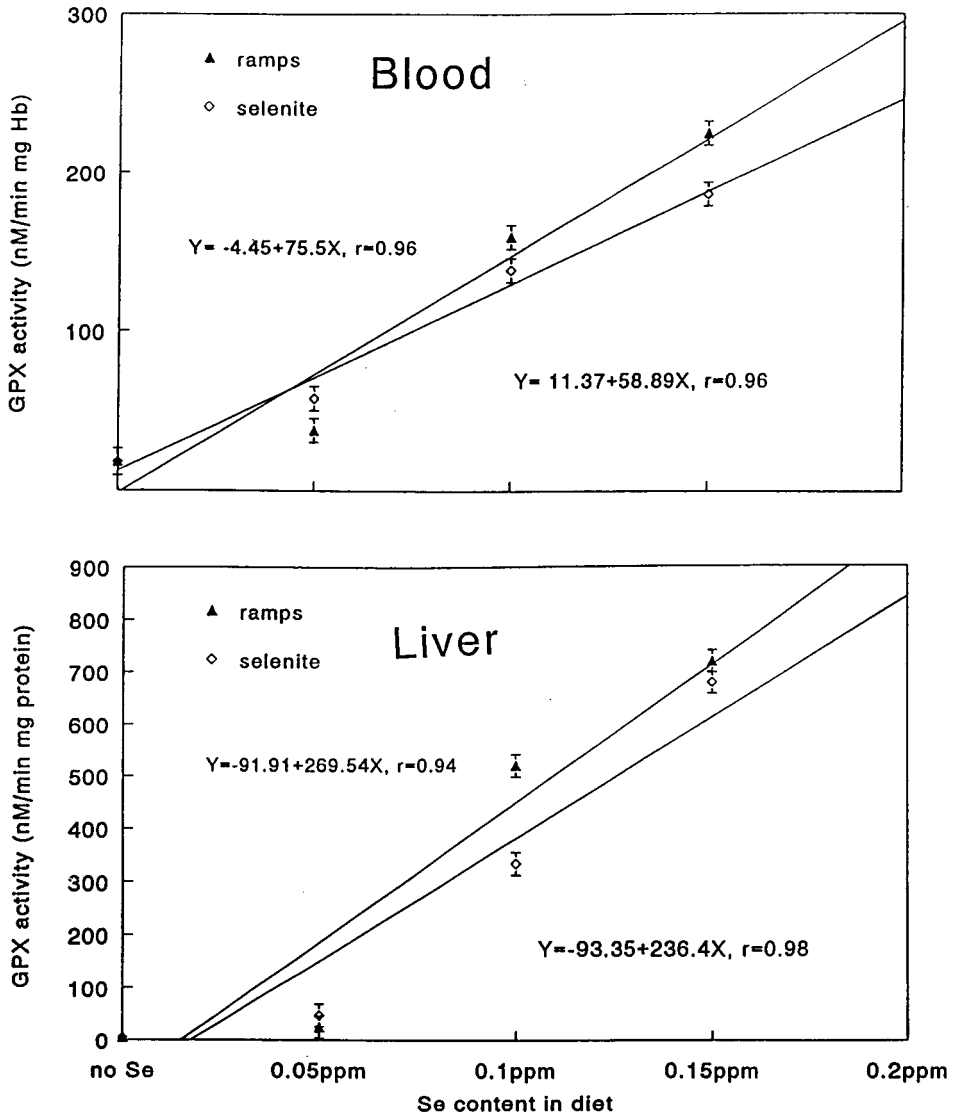

Figure 6. Regeneration of GPX activity by selenium as either selenite or Se-enriched ramps in blood or liver in Se-depleted rats. Bioavailability was determined by dividing the slope of the regression lines for the enriched ramps by those produced by selenite.

makes propagation from seeds difficult and unreliable. Ramp seeds have been suggested to exhibit an epicotyl dormancy (C. Baskin, University of Kentucky, personal communication) much like another allium, *Allium ursinum* (Ernst, 1979), and the woodland herb, *Asarum canadense* (Baskin and Baskin, 1986). Ramps have specific climatic requirements preferring acid, moist, rich soil (Vasseur and Gagnon, 1994). Bulbing occurs only in late spring apparently in a short growth phase, with most of their vegetative growth in April and May. Ramp plants cease vegetative growth in June and remain quiescent until the following spring. From a demographic study it was concluded that harvesting rates of only 5−15% were sufficient to bring the population growth rates below the equilibrium value (Nault and Gagnon, 1993), and this is the reason ramps are considered to be a "vulnerable" plant because of commercial exploitation of natural populations (Vasseur and Gagnon, 1994). In fact, in some areas of the country a permit is required to harvest ramps and there is a limitation on the number of plants that can be harvested. It is a common practice for charitable organizations to have ramp suppers in many parts of the Appalachian region, and unless methods are found to increase the production of these plants under controlled conditions, this practice may have to be curtailed. A test of the soil where the ramps grew on Cole Knob, WV, by the soil testing laboratory at Virginia Tech indicated a pH of 4.5; the phosphorus, potassium, calcium, and magnesium concentrations were, respectively, 0.5, 39, 132, and 29 mg/kg of soil, and the zinc, manganese, copper, iron, and boron concentrations were, respectively, 5, 16, 8, 67, and 0.1 mg/kg of soil. In conclusion, ramps appear to require acidic, rich, moist soil, prefer low temperature, and possibly have a day length requirement for bulbing.

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