Comparative Metabolism of Organic and Inorganic Selenium by Sheep

Jannes B. J. van Ryssen,[1] John T. Deagen, Michael A. Beilstein, and Philip D. Whanger*,[2]

Selenium (Se) and glutathione peroxidase (GPX) activity were determined in tissues of sheep fed high-Se diets with either selenite or high-Se wheat. The Se content of whole blood, but not plasma, was significantly higher in sheep fed high-Se wheat than those fed selenite. About 64% and 84% of the Se in erythrocytes were, respectively, associated with GPX in sheep fed high-Se wheat or selenite as the Se sources. Except for kidney and plasma, the Se content was higher in all tissues examined, but the percentage of Se associated with GPX was lower in tissues of sheep fed high-Se wheat than those fed selenite. Gel filtration of cytosols revealed that GPX from muscle eluted differently than that from liver. Selenocysteine was the main seleno compound in rumen microbes incubated with selenite, but selenomethionine was the predominant one when this seleno amino acid was incubated with these microbes.

The predominant chemical form of selenium (Se) in wheat has been shown to be selenomethionine (SeMet; Beilstein and Whanger, 1986a; ,Olson et al., 1970). A number of researchers have shown a difference in the metabolism of Se as selenite versus SeMet in monogastric animals (Latshaw and Osman, 1975; Osman and Latshaw, 1976; Cary et al., 1973; Latshaw and Biggert, 1981; Parsons et al., 1985; Deagen et al., 1987; Swanson, 1987). Since rumen microbes have been shown to alter the forms of Se in the rumen (Whanger et al., 1968; Hidiroglou et al., 1968), it was of interest to determine whether there would be as great a difference in the metabolism of inorganic and organic Se in ruminants as in nonruminant animals. Even though some Se is incorporated into protein, more Se is reduced to insoluble compounds when provided as selenite than as SeMet, which could influence absorption of Se from these two sources in ruminants. Therefore, Se as either selenite or high-Se wheat was fed in diets for sheep to examine possible difference in metabolism by ruminants. Based on work by others with cattle and sheep (Ullrey et al., 1977), there is reason to believe that there may be some differences in the metabolism of Se as either selenite or SeMet in ruminants.

EXPERIMENTAL PROCEDURES

Ten ewes of 8 years of age or more were divided into two groups of five each. One group was fed the diet with selenite as the source of Se and the other group Se as high-Se wheat, 1.0 μg of Se/g of diet, in each case. The ewes had a low-Se status at the start of the experiment as indicated by the blood values. The diet was composed of 70% ground alfalfa hay and 30% grain of an equal mixture of barley and corn. The high-Se wheat contained 9.8 μg of Se/g, and it was mixed with the grain mixture before addition to the hay to give a concentration of 1 mg of Se/kg on a total ration basis. The same amount of low-Se wheat (0.05 μg of Se/g) was added to the diet containing sodium selenite. After these two separate diets were thoroughly mixed, they were pelleted through a $^3/_8$-in. die. The sheep in each group were fed 9.1 kg of diet daily for 16 weeks. Blood was taken triweekly for assays and analysis.

The ewes were killed after 16 weeks, and liver, kidney, heart, pancreas, blood, muscle (semitendinosus), wool (grown over the last 8 weeks of the trial), and rumen fluid collected. The Se content was determined on each of these tissues. Wool samples were washed with distilled water and acetone before Se analysis was performed. Samples of liver, muscle, and pancreas were quickly frozen on dry ice and kept frozen (not more than 1 week) until further processing. The thawed tissues were homogenized in 0.1 M phosphate buffer, pH 6.8, and centrifuged at 12000g for 15 min, and the Se content was determined on the precipitate (pellet) and supernatant cytosol. Glutathione peroxidase (GPX) activity was assayed on a sample of the supernatant from the three tissues, and the pooled supernatant from liver and muscle of each treatment group was chromatographed on a gel filtration column (Sephadex G-150) as previously described (Beilstein and Whanger, 1986a). The eluted fractions were analyzed for Se and assayed for GPX activity. The erythrocytes were lysed with water (2 mL to 6 mL), and after centrifugation the supernatant was subjected to gel filtration. The eluted fractions were monitored for hemoglobin (540 absorbance) assayed for GPX activity and analyzed for Se.

Shortly after the sheep were killed, some rumen contents were removed and squeezed in a cotton sack, and the rumen fluid was transported to the laboratory in a styrofoam container to maintain the temperature and anaerobic conditions. The fluid was further strained through three layers of cheesecloth, with 1 volume of fluid diluted with 2 volumes of buffer (McDougall, 1948) at 39 °C, which had been saturated with carbon dioxide. Aliquots (30 mL) were transferred to 50-mL plastic centrifuge tubes containing 0.5 g of ground alfalfa and incubated under a stream of carbon dioxide for 10 min at 37 °C. Either (1 μCi) [^{75}Se]selenite or ^{75}SeMet was subsequently added and incubated at 37 °C under 1 atm of carbon dioxide for 24 h. The fluid from the sheep fed selenite was incubated with [^{75}Se]selenite, and that from those fed the high-Se wheat was incubated with ^{75}SeMet. After incubation, the fluid was centrifuged for 5 min at 1000g and the supernatant centrifuged at 12000g for 15 min to obtain the rumen microorganisms (RMO). The RMO were hydrolyzed, and the hydrolysate was chromatographed on ion-exchange columns to determine the seleno amino acids present in RMO by procedures previously noted for animal and plant tissues (Beilstein and Whanger, 1986a,b).

The remainder of the rumen contents were squeezed in a cotton sack and the RMO obtained as previously described (Whanger et al., 1978). This involved a low-speed contrifugation (750g for 5 min), followed by centrifugation in a continuous-flow centrifuge. The RMO were subsequently dried in an oven at 100 °C, ground with a micromill containing a 40-mesh screen, and analyzed for Se.

GPX was assayed by the coupled enzyme procedure (Paglia and Valentine, 1967) with the noted modifications (Whanger et al., 1977). Protein concentrations on the samples were determined with the Folin phenol reagent (Lowry et al., 1951). After digestion with nitric and perchloric acids, Se levels were determined on tissues, tissue fractions, column fractions, and RMO by a semiautomated fluorimetric method (Brown and Watkinson, 1977) using an autoanalyzer (Beilstein and Whanger, 1986a). The data were subjected to statistical analysis using the Student's t-test and calculations of correlation coefficients (Steel and Torrie, 1980).

Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331.

[1] Present address: Department of Animal Science and Poultry Science, University of Natal, Pietermaritzburg 3200, RSA.

[2] To whom all correspondence should be addressed.

0021-8561/89/1437-1358$01.50/0

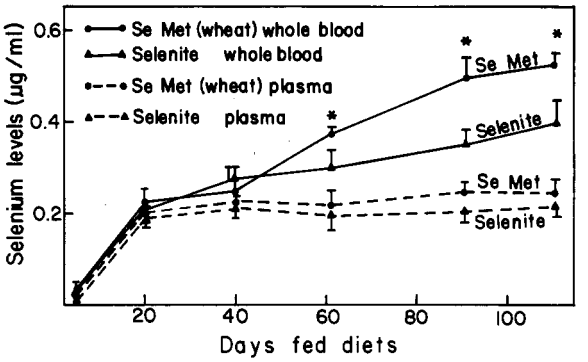

Figure 1. Accumulation of selenium in blood and plasma of sheep fed selenium as either selenite or high-Se wheat. Blood samples were taken at 0, 20, 38, 63, 91, and 111 days of feeding the two diets. Asterisk: $P < 0.05$, significantly different from erythrocytes from sheep fed selenite.

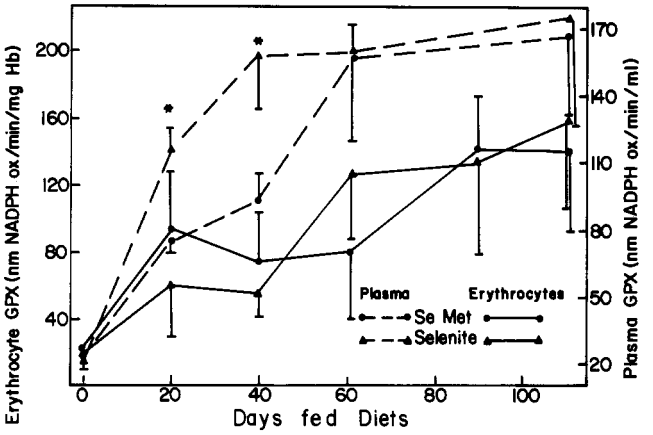

Figure 2. Erythrocyte and plasma glutathione peroxidase activities in sheep fed dietary selenium as either selenite or high-Se wheat. The blood samples were taken at the times indicated in Figure 1. Asterisk: $P < 0.05$, significantly higher than plasma from sheep fed high-Se wheat.

RESULTS

The feed was shown by analysis to contain about 1.0 mg of Se/kg for both diets. The sheep in each group consumed all of the diets, but those fed the high-Se wheat diet consumed it faster than those fed the selenite diet. For example, after 2 h all of the diet with high-Se wheat had been consumed, but about 4 kg of the selenite diet remained. Three hours after feeding, about 2.5 kg was left and there was complete consumption only after 4 h by those given the diet with selenite.

The Se content of the plasma reached a plateau after 38 days, but that for the whole blood continued to rise throughout the experiment (Figure 1). There were no differences in the Se content of the plasma between the groups at any time; however, the Se content of whole blood was significantly higher at 63, 91, and 111 days in sheep fed the high-Se wheat diet as compared to those fed the diet with selenite.

There were no differences in the erythrocyte GPX activities between the two groups (Figure 2). In general, this activity gradually increased throughout the entire time of this study and appeared to be still increasing at the end of the experiment. In contrast, plasma GPX activities increased at a much faster rate, reaching a plateau at about 40 days, and remained fairly constant thereafter. The plasma GPX activities were significantly higher at 20 and 40 days in sheep fed the diet with selenite than in those fed the high-Se wheat, but this difference had disappeared by 60 days. Calculations of correlation coefficients indicated that plasma GPX from sheep fed high-Se wheat was not as highly correlated with plasma Se levels ($r = 0.65$) as those fed selenite ($r = 0.89$). Likewise, erythrocyte GPX was not as highly correlated with whole blood selenium in sheep fed high-Se wheat ($r = 0.81$) as those fed selenite ($r = 0.87$).

Gel filtration of lysed erythrocytes revealed differences between the two treatment groups (Figure 3). There were greater amounts of Se associated with GPX in erythrocytes from the sheep fed selenite than in those fed the diet with high-Se wheat. From calculations of the area under the curves, about 84% of the Se in erythrocytes from the sheep fed selenite was associated with GPX as compared to a lower value of 64% in those from sheep fed high-Se wheat.

Except for kidney and plasma, the Se concentrations were significantly higher in all tissues in sheep fed the diet with high-Se wheat than in those fed the selenite diet (Table I). The greatest fold increase, however, of Se was in liver, muscle, and wool.

As might be expected from the Se content of the whole tissues, the Se levels in the tissue cytosols and pellets were higher in liver, pancreas, and muscle from sheep fed the high-Se wheat diet than those fed the diet with selenite (Table II). The fold increase of Se in tissue fractions from sheep fed high-Se wheat in comparison to those fed the diet with selenite ranged from 1.8 for the pancreas to a high of 4 for the liver pellets. The liver and muscle (2-fold)

Table I. Effect of Selenium either as High-Se Wheat or as Selenite upon Deposition of Selenium in Ovine Tissues[a]

tissue	diet: selenite	diet: high-Se wheat
liver	2.3 ± 0.38	6.5 ± 1.3**
kidney	9.5 ± 0.7	10.4 ± 0.8
heart	1.6 ± 0.1	2.6 ± 0.1**
pancreas	2.3 ± 0.2	3.6 ± 0.3**
muscle	0.45 ± 0.08	1.19 ± 0.19**
whole blood	0.41 ± 0.04	0.53 ± 0.03*
erythrocyte	0.72 ± 0.09	0.98 ± 0.06**
plasma	0.19 ± 0.008	0.22 ± 0.02
wool	0.45 ± 0.02	1.3 ± 0.10**
rumen microbe	2.1 ± 0.2	3.2 ± 0.2**

[a] Concentrations are given on ppm basis (dry weight), except for plasma, erythrocytes, and whole blood (wet weight). Key: *, $P < 0.05$; **, $P < 0.01$.

Table II. Selenium Content[a] in Cytosols and Pellets from Muscle, Pancreas, and Liver from Sheep Fed Diets with either Selenite or High-Se Wheat

tissue fraction	selenite	high-Se wheat[b]
liver cytosol	51 ± 8	106 ± 15**
liver pellet	352 ± 114	1434 ± 445**
pancreas cytosol	48 ± 6	85 ± 3**
pancreas pellets	264 ± 30	468 ± 31**
muscle cytosol	6 ± 2	13 ± 2**
muscle pellets	100 ± 24	319 ± 39**

[a] Micrograms of Se in fraction per gram of tissue. [b] **, significantly higher than the selenite fed sheep; $P < 0.01$.

Table III. Glutathione Peroxidase[a] Activities in Cytosols of Liver, Pancreas, and Muscle from Sheep Fed Diets with Selenite or with High-Se Wheat

tissue	diet: selenite	diet: high-Se wheat
liver	176 ± 17	178 ± 29
pancreas	449 ± 50	464 ± 111
muscle	50 ± 30	59 ± 13

[a] Activity expressed as nanomoles of NADPH oxidized per minute per milligram of protein.

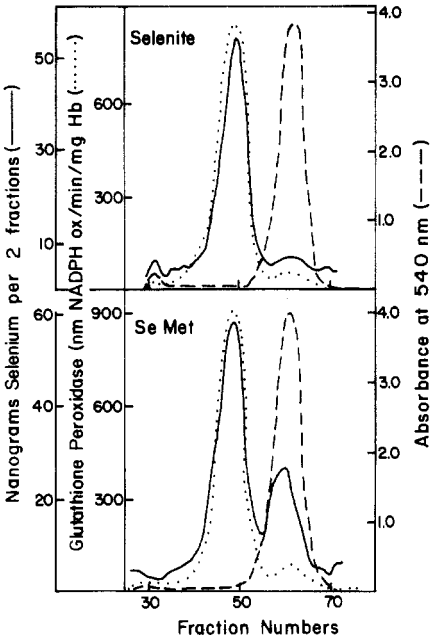

Figure 3. Gel filtration of lysed erythrocytes from sheep fed dietary selenium as either selenite or high-Se wheat. Samples from each treatment group after the diets were fed 111 days were pooled and chromatographed. A total of 6 mL was chromatographed on 2 × 120 cm columns of Sephadex G-150 with 0.05 M phosphate buffer, pH 6.3, at 4 °C at a flow rate of 12 mL/h. About 5.5 mL of effluent was collected per fraction. The selenium content, GPX activity, and hemoglobin (540 absorbance) were monitored on each of the fractions.

cytosols and muscle pellets (3-fold) were intermediate in comparison to the pancreas and liver pellet.

Even though there were significant differences in the Se content in tissues, no differences in GPX activity in liver, pancreas, or muscle were found from sheep fed diets with selenite versus high-Se wheat (Table III). This activity was highest in the pancreas, followed by the liver, with the lowest amount in the muscle.

The Se content in tissues from sheep fed the high-Se wheat diet was higher than in those from sheep fed selenite, but the GPX activity was similar. This suggested that proportionately less Se was associated with GPX in tissues from sheep fed high-Se wheat as compared to those fed selenite. Based on the data in Figure 3 where 84% of the Se was associated with GPX, the percentage of Se associated with GPX was calculated in liver, pancreas, and muscle from sheep fed selenite or high-Se wheat. The units (nanomoles of NADPH oxidized per minute) GPX per nanogram of Se for the erythrocytes from sheep fed the selenite diet at the end of the study was calculated to be 164, and this was used as the standard for calculation of the percentage of Se with GPX in the other tissues. The percentages of Se associated with GPX in liver, pancreas, and muscle from sheep fed selenite were calculated to be 24, 66, and 28, respectively, which was higher than the liver (12%), pancreas (42%), and muscle (17%) from sheep fed the high-Se wheat diet.

Only minor differences were observed between treatment groups in gel filtration patterns of liver and muscle cytosols (Figure 4). The major peak of GPX activity eluted at an earlier position in the muscle cytosols (A and B) than the main peak in the liver cytosols (C and D). The main peak of GPX activity in liver cytosols eluted at the expected position for this enzyme, but a minor peak of GPX activity eluted at the void volume of the column corresponding to the main one for the muscle. The first two Se peaks in liver cytosols from both groups of animals coeluted with the two GPX peaks. The Se peaks, however, were more pronounced in the hepatic cytosol from the sheep given selenite in the diet (Figure 4C). A minor Se peak was present in cytosol from both groups of sheep around fractions 65–68. A prominent peak of Se eluted slightly off-center from GPX in the muscle cytosol from the sheep fed high-Se wheat (Figure 4B). In contrast, the Se content in muscle cytosol from sheep fed selenite in the diet was very low, but there appeared to be minor peaks centered at fractions 42 and 50 (Figure 4A).

The uptake of ^{75}Se from [^{75}Se]selenite by RMO from sheep fed selenite ranged from 21 to 34% whereas this uptake from $^{75}SeMet$ by RMO from sheep fed high-Se wheat diet was higher, ranging from 35 to 43% (data not shown). Some rumen fluid from the sheep fed the high-Se wheat diet was also incubated with selenite and vice versa, but this did not influence the uptake patterns.

Chromatography of the hydrolysate of the RMO revealed that the ^{75}Se was present predominantly as selenocysteine when incubated with selenite but present predominantly as SeMet when incubated with SeMet (Figure 5). However, the hydrolysis recovery was much lower in the RMO incubated with selenite (51%) than those incubated with SeMet (84%).

DISCUSSION

The greater deposition of Se in tissues of ruminants fed diets naturally (organic) high in Se as compared to inorganic Se sources is consistent with work of others (Ullrey et al., 1977, 1983; Rosenfeld and Beath, 1964). This is similar to trends noted for monogastric animals (Latshaw and Osman, 1975; Cary et al., 1973; Parsons et al., 1985; Cantor et al., 1975; Whanger, 1986; Ku et al., 1972). The lack of response of the kidney to Se accumulation has been noted previously (Parsons et al., 1985), but no explanation has been offered. Both the chemical form of Se and the dietary Se levels influence the magnitude of difference between organic and inorganic Se. The differences in tissue deposition of Se from selenite versus SeMet was shown to be more pronounced as the dietary level increased (Whanger and Butler, 1988). This was particularly true in the muscle where the magnitudes of difference were 2.6, 9.0, 18, and 27 times, respectively, in rats fed 0.2, 1.0, 2.0, and 4.0 ppm Se as SeMet versus selenite.

The greater deposition of Se in tissues of ruminants given organic Se may be due in part to differences in absorption. Significantly greater absorption of Se has been shown to occur in sheep administered SeMet as compared to selenite (Peter and Whanger, 1986). One reason for this reduced absorption of Se from selenite may be because of greater reduction to insoluble compounds by RMO (Whanger et al., 1968; Peter and Whanger, 1986). The present work showing selenocysteine to be the predominant seleno amino acid when RMO were incubated with selenite and SeMet to dominate when they were incubated with SeMet is consistent with prior observations where different techniques were used to identify these seleno compounds in RMO (Whanger et al., 1968; Paulson et al., 1968).

The differences in the deposition of Se in erythrocyte fractions due to selenite versus high-Se wheat (Figure 4) were not as pronounced as found in rats (Beilstein and Whanger, 1986a; Whanger, 1986). When SeMet or high-Se wheat was given to rats, the majority of the Se in erythrocytes was associated with hemoglobin (Beilstein and Whanger, 1986a). However, in the present study only 36% of the Se in erythrocytes was associated with hemoglobin. The reason for this is not readily apparent but may be due to the longer life span of erythrocytes in sheep than rats

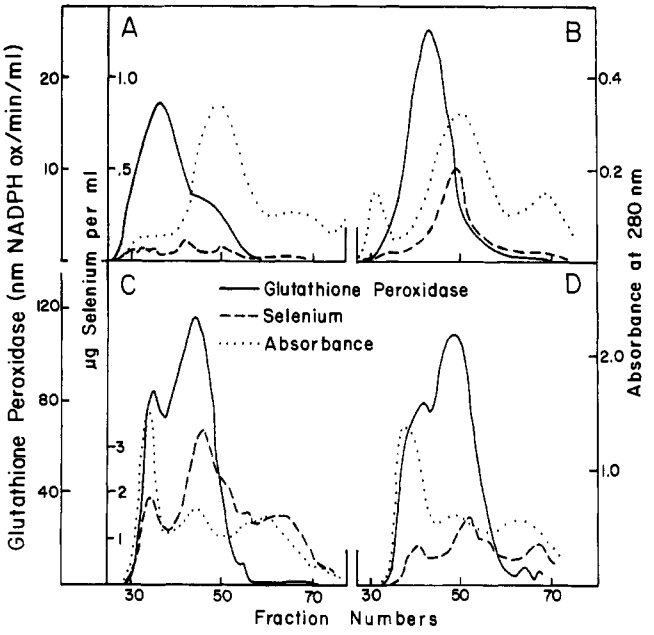

Figure 4. Gel filtration of liver and muscle cytosols from sheep fed dietary selenium as either selenite or high-Se wheat. These were pooled samples from each treatment group. The column procedures are the same as noted in Figure 3, except that 10^{-4} M dithiothreitol was included in the buffer. The fractions were monitored for selenium content, GPX activity, and protein (280 absorbance). Graph portions labeled A and B represent, respectively, muscle from sheep fed selenite or high-Se wheat, and those labeled C and D represent, respectively, liver from sheep fed selenite or high-Se wheat.

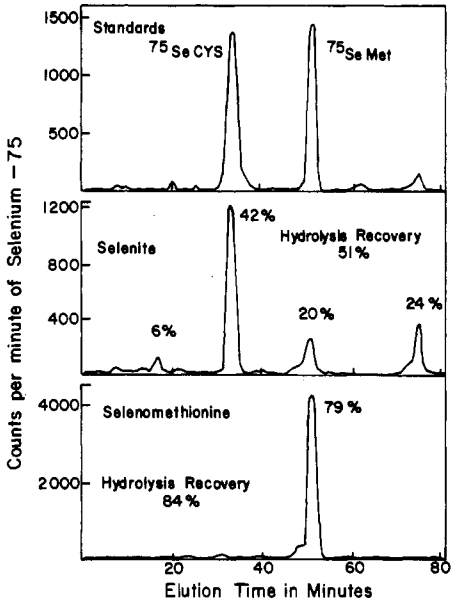

Figure 5. Chromatography of labeled seleno amino acid standards and rumen microorganism hydrolysates on Dionex DC6A resin amino acid columns. The column eluants were collected in 80 1.5-min fractions (about 0.75 mL/fraction), and ^{75}Se was determined on each fraction. The samples were counted with a Beckman Gamma 8000 counter.

(Judd and Matrone, 1962), since Se is known to be incorporated into proteins as erythrocytes are synthesized (Hafeman et al., 1974).

Selenocysteine is the major seleno amino acid in their tissue when rats are given selenite, but SeMet is usually the predominant seleno amino acid in tissues of rats given SeMet (Beilstein and Whanger, 1986b, 1988). However, in some tissues like the liver where metabolic activity is high, selenocysteine can be the main one even when SeMet is given (Beilstein and Whanger, 1986b). Attempts were made to determine the chemical forms of Se in tissues of sheep in the present study, but the levels were not high enough to detect the elution of Se from the ion-exchange columns as has been done for high-Se yeast or wheat (Beilstein and Whanger, 1986a). More Se is deposited in tissue particulate fractions of rats given SeMet than when given selenite (Beilstein and Whanger, 1988). In comparison to the tissues from sheep fed selenite, there was a 2-fold greater deposition in liver and muscle cytosols but a 3- and 4-fold greater depositions, respectively, in muscle and hepatic pellets in sheep fed the high-Se wheat (Table II). This greater fold deposition of Se in pellets than cytosols suggests the presence of higher amounts of SeMet in tissues of sheep fed high-Se wheat.

Even though GPX activity has been shown to be correlated with blood Se in animals, this is usually not true for human blood (Whanger et al., 1988). One reason for this difference may be that a greater precentage of human erythrocyte Se is associated with hemoglobin, which is in marked contrast to animal erythrocytes where most of the Se is associated with GPX (Whanger, 1986). The precentage of Se associated with GPX in human erythrocytes varies between populations (Whanger et al., 1986). Since this percentage of Se associated with erythrocyte GPX in the blood of rats (Beilstein and Whanger, 1986a) and monkeys (Butler et al., 1989) has been shown to be influenced by the dietary chemical forms, this factor would presumably have an effect on the percentage of Se associated with GPX human erythrocytes. For example, if humans consume meat from animals fed diets containing natural Se, which is likely SeMet (Olson et al., 1970; Beilstein and Whanger, 1986a), then a lower percentage of Se would become associated with erythrocyte GPX than in people consuming meat from animals fed diets with Se as selenite. Even though selenocysteine is the major seleno amino acid in tissues when animals are given selenite, selenocysteine is metabolized more like selenite than SeMet (Latshaw and Osman, 1975; Deagen et al., 1987; Cantor et al., 1975). Selenite is the predominant form of Se used in animal feed supplements and salt mixes.

The data of Table III showing no differnce in GPX activity between the treatment groups but a difference in Se content (Table I) agree with work by other researchers. Increases in tissue Se levels without a corresponding increase of GPX activities have been observed in tissues of rats (Whanger and Butler, 1988; Lane et al., 1979) and alligators (Elsey and Lance, 1983). Even though a 27-fold greater Se content was found in muscle of rats given SeMet as compared to those given selenite, no differences in GPX activity were observed (Whanger and Butler, 1988). These data along with the present work provide further evidence that the dietary forms of Se must be known to adequately interpret the data.

The differences in percentages of Se associated with tissue GPX as affected by dietary forms are consistent with earlier work from our laboratory (Beilstein and Whanger, 1988), but the percentage of cytosolic Se associated with GPX among tissues in sheep differs markedly from rat tissues. The Se associated with GPX in rat pancreas ranged from 9 to 12% (Beilstein and Whanger, 1988; Behne and Wolters, 1983), but the present study indicated a much higher amount in ovine pancreas (42–66%). Also, the muscle and liver showed diverse patterns. The percentage of Se with GPX in liver and muscle was very similar in the present study with sheep, but in rats the precentage of Se associated with GPX is 3- to 6-fold greater

in liver than in muscle (Beilstein and Whanger, 1988; Behne and Wolters, 1983).

A clear explanation cannot be offered for the gel filtration patterns of the liver and muscle cytosols (Figure 4). It is known that GPX will polymerize if a reducing agent is not included in the buffer (Stults et al., 1977). Since dithiothreitol was included in our buffers, polymerized GPX would not presumably be obtained. Apparently the small peak at the void volume of the chromatograms of hepatic cytosols and all of the muscle GPX represent polymerized GPX, assuming the molecular weight of GPX in muscle is similar to that in liver.

Previous work indicated that Se may influence the appetite of sheep (Whanger et al., 1970). When sheep were fed Se-deficient hay, those injected with Se as selenite consumed a greater portion of their ration than those not receiving parenteral Se. The present study is the only one to our knowledge demonstrating that the chemical forms of Se may affect the appetite patterns.

ACKNOWLEDGMENT

We thank Dr. O. E. Olson, Department of Chemistry, Station Biochemistry, South Dakota State University, Brooking, SD 57007, for his assistance in obtaining the high-Se wheat.

Registry No. SeMet, 3211-76-5; GPX, 9013-66-5; SeCys, 10236-58-5; Se, 7782-49-2; selenite, 15698-85-8.

LITERATURE CITED

Behne, D.; Wolters, W. Distribution of selenium and glutathione peroxidase in the rat. *J. Nutr.* **1983**, *113*, 456.

Beilstein, M. A.; Whanger, P. D. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. *J. Nutr.* **1986a**, *116*, 1701.

Beilstein, M. A.; Whanger, P. D. Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine. *J. Nutr.* **1986b**, *116*, 1711.

Beilstein, M. A.; Whanger, P. D. Glutathione peroxidase activity and chemical forms of selenium in tissues of rats given selenite or selenomethionine. *J. Inorg. Biochem.* **1988**, *33*, 31.

Brown, M. W.; Watkinson, J. H. An automated fluorimetric method for the determination of nanogram quantities of selenium. *Anal. Chim. Acta* **1977**, *89*, 29.

Butler, J. A.; Whanger, P. D.; Kaneps, A. J.; Patton, N. M. Metabolism of selenite and selenomethionine in the rhesus monkey (*Macaca mulatta*). *J. Nutr.* **1989**, submitted for publication.

Cantor, A. H.; Langevin, M. L.; Noguchi, T.; Scott, M. Efficacy of selenium in selenium compounds and feedstuffs for prevention of pancreatic fibrosis in chicks. *J. Nutr.* **1975**, *105*, 106.

Cary, E. E.; Allaway, W. H.; Miller, M. Utilization of different forms of dietary selenium. *J. Anim. Sci.* **1973**, *36*, 285.

Deagen, J. T.; Butler, J. A.; Beilstein, M. A.; Whanger, P. D. Effects of dietary selenite, selenocysteine and selenomethionine on selenocysteine lyase and glutathione peroxidase activities and on selenium levels in rat tissues. *J. Nutr.* **1987**, *117*, 91.

Elsey, R. M.; Lance, V. Effect of diet on blood selenium and glutathione peroxidase activity in the alligator. *Comp. Biochem. Physiol.* **1983**, *76B*, 831.

Hafeman, D. G.; Sunde, R. A.; Hoekstra, W. G. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *J. Nutr.* **1974**, *104*, 580.

Hidiroglou, M.; Heaney, D. P.; Jenkins, K. J. Metabolism of inorganic selenium in rumen bacteria. *Can J. Physiol. Pharmacol* **1968**, *46*, 229.

Judd, J. T.; Matrone, G. Sheep erythrocyte life span in estimation of hemoglobin turnover in iron metabolism studies. *J. Nutr.* **1962**, *77*, 264.

Ku, P. K.; Ely, W. T.; Groce, A. W.; Ullrey, D. E. Natural dietary selenium, α-tocopherol and effect on tissue selenium. *J. Anim. Sci.* **1972**, *34*, 208.

Lane, H. W.; Shirley, R. L.; Cerda, J. J. Glutathione peroxidase activity in intestinal and liver tissues of rats fed various levels of selenium, sulfur and alpha-tocopherol. *J. Nutr.* **1979**, *109*, 444.

Latshaw, J. D.; Osman, M. Distribution of selenium in egg white and yolk after feeding natural and synthetic selenium compounds. *Poult. Sci.* **1975**, *54*, 1244.

Latshaw, J. D.; Biggert, M. D. Incorporation of selenium into egg proteins after feeding selenomethionine or sodium selenite. *Poult. Sci.* **1981**, *60*, 1309.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265.

McDougall, E. I. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochem. J.* **1948**, *43*, 99.

Olson, O. E.; Novacek, E. J.; Whitehead, E. I.; Palmer, I. S. Investigations on selenium in wheat. *Phytochemistry* **1970**, *81*, 1188.

Osman, M.; Latshaw, J. D. Biological potency of selenium from sodium selenite, selenomethionine, and selenocystine in the chick. *Poult. Sci.* **1976**, *55*, 987.

Paglia, D. E.; Valentine, W. N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **1967**, *70*, 158.

Parsons, M. J.; Ku, P. K.; Ullrey, D. E.; Stowe, H. D.; Whetter, P. A.; Miller, E. R. Effects of riboflavin supplementation and selenium source on selenium metabolism in the young pig. *J. Anim. Sci.* **1985**, *60*, 451.

Paulson, G. D.; Baumann, C. A.; Pope, A. L. Metabolism of ^{75}Se-selenite, ^{75}Se-selenate, ^{75}Se-selenomethionine and ^{35}S-sulfate by rumen microorganisms in vitro. *J. Anim. Sci.* **1968**, *27*, 497.

Peter, D. W.; Whanger, P. D. Metabolism of selenite and selenomethionine by sheep. CSIRO Division of Animal Production, Wembley, Western Australia, 1986, unpublished observation.

Rosenfeld, I.; Beath, O. A. *Selenium: Geobotany, Biochemistry, Toxicity and Nutrition*; Academic Press: New York, 1964; 410 pp.

Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of statistics*, 2nd ed.; McGraw-Hill: New York, 1980.

Stults, F. H.; Forstrom, J. W.; Chiu, D. T. Y.; Tappel, A. L. Rat liver glutathione peroxidase: Purification and study of multiple forms. *Arch. Biochem. Biophys.* **1977**, *183*, 490.

Swanson, C. A. Comparative utilization of selenite, selenomethionine, and selenized yeast by the laying hen. *Nutr. Res.* **1987**, *7*, 529.

Ullrey, D. E.; Brady, P. S.; Whetter, P. A.; Ku, P. K.; Magee, W. T. Selenium supplementation of diets for sheep and beef cattle. *J. Anim. Sci.* **1977**, *45*, 559.

Ullrey, D. E.; Combs, G. F., Jr.; Conrad, H. R.; Hoekstra, W. G.; Jenkins, K. J. W.; Levander, O. A.; Whanger, P. D. *Selenium in Nutrition*; National Academy Press: Washington, DC, 1983; p 57.

Whanger, P. D. Some comparative aspects of selenite and selenomethionine metabolism. *J. Am. Coll. Toxicol.* **1986**, *5*, 101.

Whanger, P. D.; Butler, J. A. Effects of various dietary levels of selenium as selenite or selenomethionine on tissue selenium levels and glutathione peroxidase activity in rats. *J. Nutr.* **1988**, *118*, 846.

Whanger, P. D.; Weswig, P. H.; Muth, O. H. Metabolism of ^{75}Se-selenite and ^{75}Se-selenomethionine by rumen microorganisms. *Fed. Proc.* **1968**, *27*, 418.

Whanger, P. D.; Weswig, P. H.; Muth, O. H.; Oldfield, J. E. Selenium and white muscle disease: Effect of sulfate and energy levels on plasma enzymes and ruminal microbes. *Am. J. Vet. Res.* **1970**, *31*, 965.

Whanger, P. D.; Weswig, P. H.; Schmitz, J. A.; Oldfield, J. E. Effects of selenium and vitamin E on blood selenium levels, tissue glutathione peroxidase activities and white muscle disease in sheep fed purified or hay diets. *J. Nutr.* **1977**, *107*, 1298.

Whanger, P. D.; Weswig, P. H.; Oldfield, J. E. Selenium, sulfur and nitrogen levels in ovine rumen microorganisms. *J. Anim. Sci.* **1978**, *46*, 515.

Whanger, P. D.; Robinson, M. F.; Feldman, E. B.; Beilstein, M. A.; Butler, J. A. Variations in the distribution of selenium

between erythrocyte glutathione peroxidase and hemoglobin in different human populations. *Fed. Proc.* **1986**, *45*, 474.
Whanger, P. D.; Beilstein, M. A.; Thomson, C. D.; Robinson, M. F.; Howe, M. Blood selenium and glutathione peroxidase activity of populations in New Zealand, Oregon and South Dakota. *FASEB J.* **1988**, *2*, 2996.

Received for review November 28, 1988. Accepted April 25, 1989. Published with the approval of the Oregon State University Experiment Station as Technical Paper No. 8708. This study was supported in part by Public Health Service Research Grant DK 38306 from the National Institute of Diabetes and Digestive and Kidney Diseases.

Isolation of Compounds with Antimutagenic Activity from Savoy Chieftain Cabbage

Terence Lawson,* Janet Nunnally, Betty Walker, Edward Bresnick, Desmond Wheeler, and Maureen Wheeler

Dried leaves of Savoy Chieftain cabbage (*Brassica oleracea*) were extracted with methanol, methylene chloride, or petroleum ether. Soluble extracts were fractionated by flash chromatography on silica gel columns. Nonacosane, 15-nonacosanone, pheophytin *a*, and β-sitosterol were isolated and examined for their ability to inhibit the mutagenicity of *N*-methyl-*N*-nitrosourea (MNU) and 2-aminoanthracene (2-AA) in the Ames bacterial and V79 cell mammalian mutagenicity assays. In the Ames assay, nonacosane and pheophytin were without inhibitory action against either 2-AA- or MNU-induced mutagenesis. 15-Nonacosanone was more effective than β-sitosterol. In the V79 assay, all four compounds were active against the mutagenicity of 2-AA but only nonacosane, 15-nonacosanone, and β-sitosterol were active against that of MNU. Other crude extracts were identified with antimutagenic activity, but their compositions were not determined.

Epidemiological data have shown an inverse relationship between vegetable consumption and the relative risk of developing colon and stomach cancers (Graham et al., 1972; Modan et al., 1975). A lower incidence of stomach cancer occurred in nonsmokers who consistently ate vegetables than in those who did not (Hirayama, 1977). An inverse relationship between the consumption of cruciferous vegetables (e.g., cabbage) and the incidence of colon (Graham et al., 1978), stomach (Haenszel et al., 1976), and breast and prostatic (Phillips, 1975) cancers has been shown.

Laboratory studies have shown that vegetables or their extracts can inhibit carcinogenesis and interfere with the metabolism and mutagenicity of carcinogens (Birt and Bresnick, 1988). Addition of naturally occurring indoles to the diet reduced the incidences of 7,12-dimethylbenz[*a*]anthracene (DMBA) induced mammary and 3,4-benzo[*a*]pyrene (BP) induced gastric tumors by at least 55% (Wattenberg and Loub, 1978). Cinnamic acid derivatives (Wattenberg, 1983) and flavonoid-like compounds (Sparnins et al., 1982) reduced the incidence of chemically induced tumors. Many naturally occurring compounds act as antioxidants (Sparnins et al., 1982) or stimulate glutathione (GSH) synthesis or the levels of those compounds involved in maintaining GSH levels. A positive effect on these processes would facilitate the formation of GSH derivatives of the electrophiles of chemical carcinogens, representing a major detoxification pathway. Coffee bean extracts induced GSH *S*-transferase activity in vivo (Lam et al., 1982). A diet supplemented with broccoli stimulated GSH *S*-transferase and arylhydrocarbon hydroxylase (AHH) activities in rat liver and significantly altered the profile of BP metabolites (Aspry and Bjeldanes, 1983). Diets containing cabbage, Brussels sprouts, or alfalfa similarly affected BP metabolism in mouse liver (Hendrich and Bjeldanes, 1983). AHH activity was stimulated by plant phenols, leading to changes in BP metabolism by epidermal microsomal preparations (Das et al., 1987).

The mutagenicities of BP and 3-methylcholanthrene (3-MC) (Lai et al., 1980), DMBA, MNU, BP, and 4-nitroquinoline oxide (Kimm et al., 1982), and BP and a cigarette smoke condensate (Terwel et al., 1985) were reduced by vegetable extracts. The reduction correlated with the chlorophyll content of the extracts. An antimutagenic component of these extracts was identified as chlorophyllin (Lai et al., 1980; Kimm et al., 1982; Ong et al., 1986).

In the present study, dried leaves of Savoy Chieftain cabbage were extracted with organic solvents. Each extract was tested for antimutagenic activity in a mammalian mutagenicity (Jenssen, 1984) system using 2-aminoanthracene (2-AA) (indirect acting) and MNU (direct acting) as mutagens.

MATERIALS AND METHODS

Animals. Eight-week-old male Syrian golden hamsters [Unei(SYR)] from the Eppley Institute colony were used as a source of hepatocytes in the V79 assay.

Isolation of Extracts. Savoy Chieftain cabbage (*Brassica oleracea*), grown by the Department of Horticulture, University of Nebraska, was dried by either (i) air-drying individual leaves at 4 °C, (ii) forced air-drying of individual leaves at 40 °C, or (iii) freeze-drying shredded cabbage. The type of drying did not affect the nature of the extracts. The harvested material was about 7% of the wet weight. Dried cabbage was stored at −20 °C until extracted. Of the dried cabbage 30–50 g was exhaustively extracted with petroleum ether, methylene chloride, or methanol in a Soxhlet, yielding 1.6%, 1.0%, and 7.6% of the starting material, respectively. These extracts were subjected to further organic extractions, typical examples of which are described below.

(*a*) *Petroleum Ether Extracts.* The dried petroleum ether extract was dissolved in warm acetone and the resultant mixture cooled and filtered to remove some of the lipids. Acetone was

Eppley Institute for Research in Cancer and Allied Diseases (T.L., J.N., B.W., E.B.), Department of Pharmaceutical Sciences (T.L.), and Departments of Biochemistry and Pharmacology (E.B.), University of Nebraska Medical Center, Omaha, Nebraska 68105–1065, and Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588 (D.W., M.W.).

0021-8561/89/1437-1363$01.50/0