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Uptake of selenite, selenomethionine and selenate by brush border membrane vesicles isolated from rat small intestine

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The uptake of selenite, selenate and selenomethionine (SeMet) was performed with brush border membrane vesicles (BBMV) prepared from rats fed selenium-deficient and supplemented diets. At equilibrium (60 min), the uptake of ^{75}Se from [^{75}Se]selenite ranged from 16.5 to 18.9 nmol mg^{-1} protein. There was a curvilinear relationship in the uptake of selenite over a concentration range of 10–1000 μM. About 2 nmol mg^{-1} protein was obtained with selenomethionine (SeMet) which occurred between 90 and 180 s. In contrast to selenite, there was a linear relationship in the initial uptake of SeMet over a concentration range of 10–1000 μM. The uptake of selenate was approximately 50-fold lower than selenite, reaching 350 pmol mg^{-1} protein. Dietary selenium level had no effect on the rate of ^{75}Se accumulation by BBMV. Dramatic differences are found in the uptake and binding of selenium by BBMV incubated with different selenocompounds.

Keywords: brush border membrane vesicles, rat small intestine, selenate, selenite, selenomethionine

Introduction

Several different studies with selenium have been conducted with brush border membrane vesicles (BBMV). Purified BBMV have been used extensively to examine the role of the microvillar membrane in the process of absorption in isolation from subsequent metabolic and transport events carried out by the enterocyte. Previous research with selenium includes the study of the transport of selenate and selenite by membrane vesicles from rat and sheep small intestine (Arduser *et al.* 1986), uptake of selenate and selenite by membrane vesicles from pig, sheep and rat small intestine (Wolffram *et al.* 1986), transport of selenate and sulfate across membranes from pig jejunum (Wolffram *et al.* 1988), stimulation of selenium uptake from selenite by L-cysteine in sheep small intestine (Wurmli *et al.* 1989), the effects of vitamin D status on uptake by BBMV of chicks (Mykkanen & Wasserman 1989), and the effect of methionine on transport of selenomethionine (SeMet) by membrane vesicles from pig jejunum (Wolffram *et al.* 1989).

Many investigators have found a high affinity of selenite and selenomethionine (SeMet) for sites on the brush border membrane (Wolffram *et al.* 1986, 1989, Mykkanen & Wasserman 1989). Because the luminal membrane provides entry for uptake by cellular transport processes, the interaction between various selenocompounds and BBMV may shed light on the mechanisms of absorption. As yet, no transport system has been identified which primarily and specifically exists for the intestinal uptake of selenium.

Information is unavailable on the effect of diet on selenium uptake by BBMV. However, *in vivo* studies indicate no effect of selenium status on selenium absorption. Balance studies with rats (Brown *et al.* 1972), and *in vivo* absorption measurements with chick duodenum (Humaloja & Mykkanen 1986) and rat small intestine (Vendeland *et al.* 1992a) provide no evidence for an effect of selenium status on this process. Interestingly, vitamin D deficiency reduced the ability of chick duodenal BBMV to bind selenite (Mykkanen & Wasserman 1989), suggesting some dietary components could affect selenium absorption.

The purpose of the present studies was to compare the uptake characteristics of three different selenium compounds by BBMV under identical experimental conditions. The uptake of several selenium compounds has been studied under various conditions (Wolffram *et al.* 1985, 1986, Arduser *et al.* 1986, Mykkanen & Wasserman 1989), but reports are unavailable where selenite, selenate and SeMet have been investigated together under similar conditions. The effects of dietary selenium deficiency and

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supplementation with inorganic or organic selenium on uptake by BBMV were also investigated.

Methods and materials

Animals and diets

Male Sprague-Dawley rats weighing initially about 150 g (Simonsen, Gilroy, CA) were given *ad libitum* access to tap water and either a purified diet or commercial rat chow (Wayne Lab-blox, Allied Mills, Chicago, IL). The purified diet was composed of 30% torula yeast (Rhinelander Paper, Rhinelander WI), 51.5% sucrose, 9% solka floc, 5% corn oil, 3.5% mineral and 1% vitamin mixes (American Institute of Nutrition 1977) and 0.3% D,L-methionine. The torula yeast furnished adequate choline to meet the requirements of the rat (2.5–3.0 mg g^{-1}). Selenium analysis indicated that the diets contained (in mg Se kg^{-1} diet) purified basal, 0.02; sodium selenite-supplemented, 0.20; SeMet-supplemented, 0.20; and rat chow, 0.46.

Procedures

Twelve rats were fed each of the purified diets for at least 8 weeks to allow sufficient time for the production of selenium deficiency. Unfasted animals were anesthetized with sodium pentobarbital (80 mg kg^{-1}). A midline laparotomy was made and the small intestine exposed. An intraduodenal cannula was inserted for the introduction of ice cold Earle's Balanced salt solution (Earle 1943). Simultaneously, the external surface of the intestine was bathed in the same solution. These measures were undertaken to minimize mucosal cell loss. The entire small intestine was removed and placed in fresh ice-cold Earle's salt solution. Samples of liver, muscle and whole blood were taken for measurement of selenium. The intestine was slit longitudinally, blotted gently and the mucosa was scraped from the underlying muscle layers with a glass microscope slide. The mucosal scrapings from the entire small intestine were weighed and homogenized in 20 volumes (20 ml g^{-1}) of isolation medium, 0.1 M D-mannitol and 10 mM Tris–HEPES, pH 7.5, for 3 min at maximum speed in an Omni-mixer (Sorvall, New Bedford, CT).

The BBMV fraction was isolated from this tissue preparation as described by Muir *et al.* (1984). Magnesium chloride was added to achieve a concentration of 20 mM and the homogenate was stirred for 30 min at 4 °C. The precipitated material was separated by centrifugation for 10 min at 4 °C at 6000 × *g*. The supernate was centrifuged at 21 000 × *g* for 30 min, the pellet suspended in 10 volumes (10 ml g^{-1}) of the isolation medium and homogenized with a glass–teflon homogenizer for six strokes at 1000 r.p.m. A solution of anhydrous magnesium sulfate (12 mg ml^{-1} in isolation medium) was added to bring the final concentration to 0.1 mM. The suspension was centrifuged at 6000 × *g*. The pellet was discarded and brush borders collected by centrifuging the supernate at 21 000 × *g* for 25 min. The pellet was resuspended in 10 volumes (10 ml g^{-1}) of suspension solution (0.1 M mannitol, 10 mM Tris–HEPES and 0.1 mM magnesium sulfate) by passage through a 27 gauge needle. The suspension was centrifuged at 2000 × *g* for 5 min. The supernate was centrifuged at 48 000 × *g* for 15 min (model L2–65; Beckman, Fullerton, CA) and the pellet was resuspended in suspension solution to a final protein concentration of 3–6 mg ml^{-1}. Uptake assays were either begun immediately or after storing in liquid nitrogen as previously described (Stevens *et al.* 1982).

Selenium levels in diets, liver and muscle samples and whole blood were determined fluorimetrically according to the semi-automated method of Brown & Watkinson (1977), using an Alpkhem II system (Alpkhem, Milwaukie, OR). Whole blood glutathione peroxidase (GSHPx, EC 1.11.1.9) activity was determined by the coupled enzyme procedure of Paglia & Valentine (1967) as modified by Black *et al.* (1978). The purity of the BBMV fraction was assessed from the enrichment of the brush border marker enzyme, sucrase, over the concentration in the homogenate. Sucrase was measured by the method of Dahlqvist (1968).

Protein determination

Aliquots of tissue homogenates and membrane preparations were precipitated with 10% trichloroacetic acid and dissolved in 0.5 N NaOH at 37 °C. Following neutralization with 0.5 N HCl, protein was analyzed according to Lowry *et al.* (1951). Bovine serum albumin served as the reference protein.

Uptake measurements

The functional integrity of the membrane vesicles was evaluated by their ability to support concentrative uptake of D-glucose under Na^+ gradient conditions at 25 °C. The final concentrations of the components of the reaction mixture were 100 μM D-glucose plus tracer D-[^{3}H]glucose (40 μCi ml^{-1}), 0.1 mM mannitol, 10 mM Tris–HEPES, pH 7.5, 0.1 M NaCl or NaSCN and approximately 2 mg ml^{-1} membrane protein. At various times, aliquots representing 50 μg protein were removed and diluted into 50 volumes of ice-cold stop solution (0.9% NaCl, 10 mM Tris–HEPES and 0.2 mM phlorizin), rapidly filtered (Millipore filters, HA025; sampling manifold, model 1225; Millipore, Bedford, MA), and washed with 3.75 ml stop solution. Filters were dissolved in 0.5 ml 1 N HCl followed by 1 ml ethyl acetate. Radioactivity was measured by liquid scintillation counting in a Packard Tri-Carb 4530 instrument.

The uptake of selenium compounds was assayed in an incubation mixture at 37 °C, pH 7.5, with final concentrations of 10–1000 μM selenium containing tracer [^{75}Se]selenite, [^{75}Se]SeMet or [^{75}Se]selenate (0.45 μCi ml^{-1}), 100 mM mannitol, 10 mM Tris–HEPES, 0.1 mM $MgSO_4$. Membrane concentrations were 2–3 mg ml^{-1} during SeMet and selenate uptake but were diluted to approximately 0.25 mg ml^{-1} for uptake studies of selenite. Radioactivity was measured in a gamma scintillation counter

(Beckman Instruments Model 8000). Mannitol, at increasing concentrations, served as the osmotic agent for binding studies. The stop solution consisted of 0.9% NaCl and 10 mM Tris–HEPES, pH 7.5.

Controls to correct for non-specific binding of tracers to filter paper were used during every experiment. Aliquots of the reaction mixture minus D-glucose or the appropriate selenium compound were diluted into the stop solution. Substrate plus tracer were added and the solution filtered and washed as described above.

During the selenium uptake studies, valinomycin and K^+ (100 mM KCl) were added when a Na^+ gradient was used to avoid the formation of an artificial diffusion potential across the membrane which could erroneously suggest sodium-dependent transport. Nigericin was added to counteract a pH gradient generated by Na^+/H^+ countertransport known to take place in intestinal brush border membranes. The low pH at the external membrane surface could result in the permeation of the uncharged selenium species, thus erroneously indicating coupling to sodium transport (Murer & Kinne 1980). When valinomycin and nigericin were added, they were present at final concentrations of 0.5 μM. The ^{75}Se-labeled selenocompounds were obtained from Amersham (Arlington Heights, IL).

Statistical analysis

Statistical analyses were performed with the Student–Neuman–Keuls test (Steel & Torrie 1980). A value of $P < 0.05$ was considered statistically significant.

Results

The selenium status of the experimental rats was confirmed by the tissue selenium content and whole blood GSHPx activity (Table 1). Whole blood selenium levels in deficient rats were 20% of those in animals fed either selenite or SeMet ($P < 0.01$). Liver selenium levels were 7 and 128% for deficient and SeMet-fed rats, respectively ($P < 0.01$) as compared with liver selenium levels of selenite-fed animals. Selenium deprivation lowered muscle selenium to 39% of levels achieved by selenite-fed rats while rats supplemented with SeMet reached tissue levels 179% of the selenite fed rats ($P < 0.01$). Whole blood GSHPx activity in selenium-deficient rats was 22% of that measured in rats fed the supplemented diets ($P < 0.01$).

The purity of the BBMV preparation was assessed by the enrichment of the brush border enzyme, sucrase. Diet had no statistically significant effect on either the purification or recovery of sucrase activity (data not shown). Specific activity of sucrase (mean ± SEM) in the brush border fraction (0.657 ± 0.024 μmol min^{-1} mg^{-1} vesicular protein) was increased an average of 17-fold (17.4 ± 0.9) over the mucosal homogenate (0.039 ± 0.002 μmol min^{-1} mg^{-1}) and the recovery was 32% (32.2 ± 1.3).

Diet had an influence on the amount of mucosal tissue obtained and the final yield of protein in the brush border fraction (Table 2). The amount of mucosal scrapings from the deficient rats was 9% higher than the amount of tissue from the rats fed selenite ($P < 0.05$). In contrast, the final recovery of homogenate protein in the BBMV fraction was 22% greater from the mucosa of rats fed selenite than for the SeMet-fed animals ($P < 0.05$).

The ability of the BBMV to transport glucose is demonstrated in Figure 1. Glucose uptake into the intravesicular space was rapid and reached an equilibrium value of 110 pmol mg^{-1} protein within approximately 5 min.

When an inwardly directed Na^+ gradient (100 mM) was present, glucose was actively transported and transiently accumulated to a level approximately 9-fold higher than the equilibrium value. With the rapid dissipation of the gradient, intravesicular glucose concentration returned to

Table 1. Tissue selenium and whole blood glutathione peroxidase activity levels

	Liver selenium (ng g^{-1} dry wt)	Muscle selenium (ng g^{-1} dry wt)	Blood selenium (ng ml^{-1})	Blood GPX (nm min^{-1} mg Hb^{-1})
Selenium deficient	$156^a \pm 9$	$181^a \pm 11$	$90^a \pm 7$	$106^a \pm 8$
Selenite supplemented	$2230^b \pm 83$	$466^b \pm 15$	$449^b \pm 17$	$491^b \pm 31$
SeMet supplemented	$2849^c \pm 107$	$833^c \pm 26$	$480^b \pm 9$	$499^b \pm 16$

Values are means ± SEM for 12 animals (final body weight averaged approximately 250 g). Means with different superscripts for each analysis are significantly different. $^{a,b,c}P < 0.05$, $^{e,f,g}P < 0.01$.

Table 2. Yield of mucosal tissue, homogenate protein and brush border membrane protein during BBMV preparation from rats fed selenium-deficient and -supplemented diets

	g mucosa/rat	g homogenate protein/ g mucosa protein	mg brush border protein/ g mucosal tissue	mg brush border protein/ mg homogenate protein
Selenium deficient	$3.37^a \pm 0.16$	$0.117^a \pm 0.017$	$2.15^a \pm 0.39$	$0.0182^{ab} \pm 0.002$
Selenite supplemented	$2.84^b \pm 0.17$	$0.122^a \pm 0.009$	$2.56^a \pm 0.14$	$0.0210^a \pm 0.001$
SeMet supplemented	$2.98^{ab} \pm 0.12$	$0.126^a \pm 0.007$	$2.17^a \pm 0.25$	$0.0172^b \pm 0.002$

Values are means ± SEM for four preparations of BBMV. ^{a,b}Values with different superscripts are significantly different at $P < 0.05$.

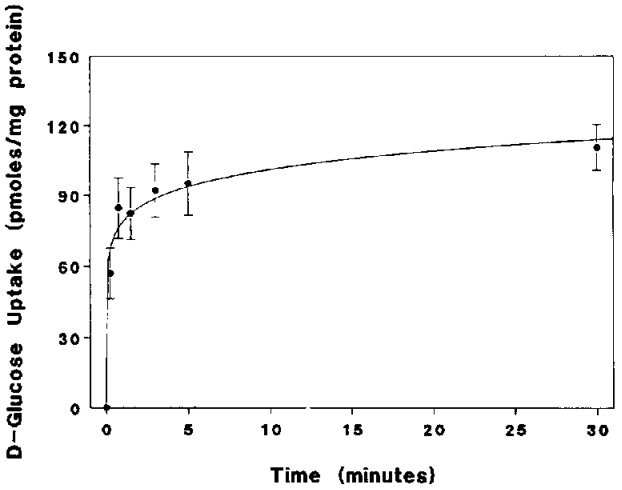

Figure 1. Glucose uptake by BBMV from rats fed a selenium-adequate diet. Uptake of 100 mM D-glucose plus tracer (D-[^{3}H]glucose, 2 μCi) into BBMV equilibrated in an incubation medium at 25 °C containing 100 mM mannitol, 10 mM Tris–HEPES, 100 mM NaCl and 2 mg ml^{-1} vesicular protein, pH 7.5. Each point represents the mean ± SEM for three experiments.

equilibrium levels within 5 min (Figure 2). Similar results were observed for freshly prepared vesicles and those stored in liquid nitrogen for up to 1 week. The approximate intravesicular volume calculated from the equilibrium uptake of glucose was 1.1 μl mg^{-1} protein.

The uptake of ^{75}Se as selenate under Na$^+$-equilibrated conditions over a 90 min period is shown in Figure 3. Rapid uptake occurred during the first 3 min with a slower uptake that never reached equilibrium over the entire 90 min. However, this does appear to be approaching a plateau near 350 pmol mg^{-1} protein. Dietary selenium did not influence the time course of uptake (data not shown).

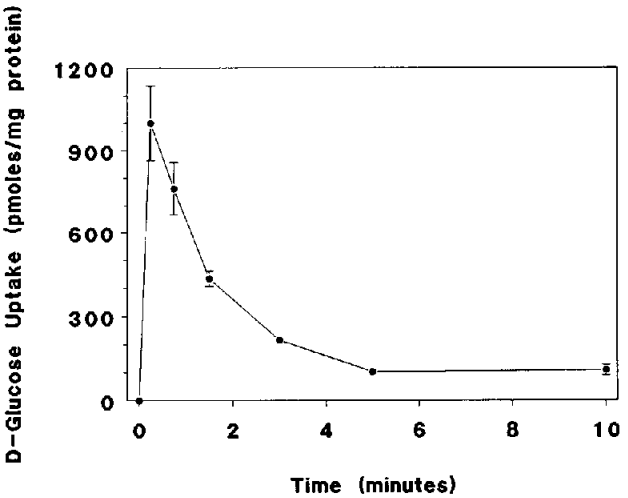

Figure 2. Concentrative uptake of D-glucose by BBMV from rats fed a selenium-adequate diet was fueled by a 100 mM Na$^+$ gradient. The incubation mixture contained 100 mM mannitol, 10 mM Tris–HEPES and 2 mg ml^{-1} vesicular protein, pH 7.5. NaSCN was added extravesicularly in place of the equilibrated NaCl. Each point represents the mean ± SEM for three experiments.

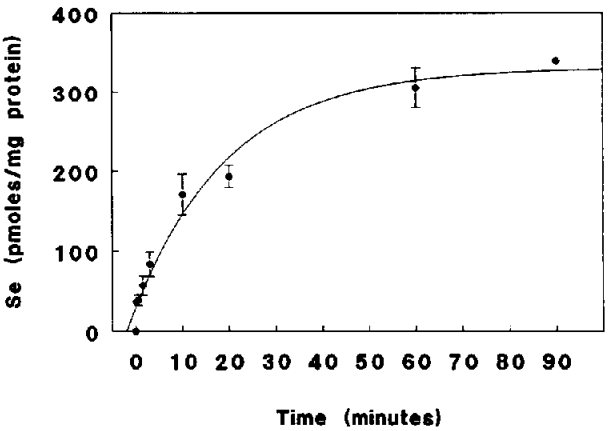

Figure 3. Selenium uptake from [^{75}Se]selenate by rat BBMV prepared from animals fed selenium-adequate diets. The incubation mixture at 37 °C contained 100 mM mannitol, 100 mM NaCl, 10 mM Tris–HEPES, 0.1 mM $MgSO_4$, 2–3 mg ml^{-1} vesicular protein, pH 7.5 to which 50 μM selenate plus tracer ([^{75}Se] selenate, 0.45 μCi ml^{-1}) was added. Data are expressed as the mean ± SEM for seven experiments.

The uptake of [^{75}Se]selenate over a concentration range of 10–1000 μM at 1 min is shown in Figure 4. The uptake rises in linear fashion over the entire concentration range with no indication of saturation.

The uptake of ^{75}Se as SeMet by BBMV from rats fed selenium-deficient and supplemented diets under Na$^+$-equilibrated conditions is shown in Figure 5. Regardless of diet, [^{75}Se]SeMet is taken up rapidly and approaches equilibrium near approximately 2.4 nmol mg^{-1} protein. This value is approximately 7-fold higher than the equilibrium uptake of selenate. Although expected, stimulation of uptake in the presence of an inwardly directed Na$^+$ gradient could not be demonstrated.

Figure 6 shows the initial uptake (30 s) of [^{75}Se]SeMet over a concentration range of 10–1000 μM. Data from all

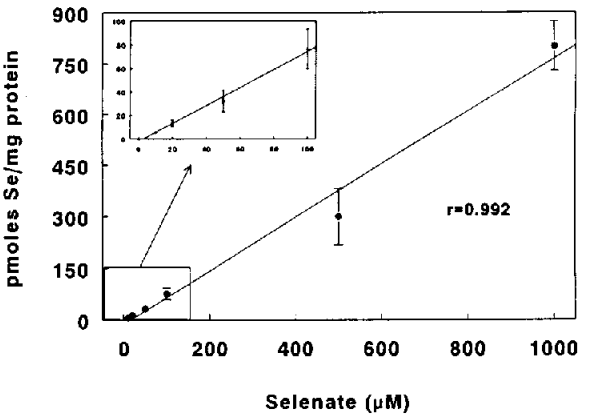

Figure 4. Selenium uptake from [^{75}Se]selenate by BBMV over the concentration range 10–1000 μM after 1 min of incubation. BBMV were prepared from rats fed selenium-adequate diets. The incubation mixture at 37 °C contained 100 mM mannitol, 100 mM NaCl, 10 mM Tris–HEPES, 0.1 mM $MgSO_4$, 2–3 mg ml^{-1} vesicular protein, pH 7.5. Data are expressed as the mean ± SEM for four experiments.

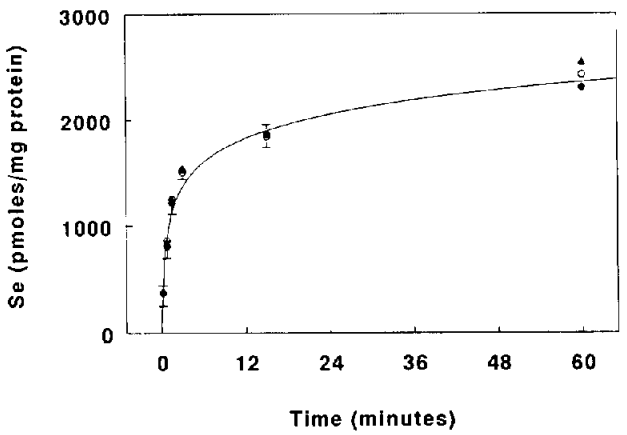

Figure 5. Selenium uptake from [^{75}Se]SeMet by rat BBMV prepared from animals fed selenium-deficient and diets supplemented with either selenite or SeMet. The incubation mixture at 37 °C contained 100 mM mannitol, 100 mM NaCl, 10 mM Tris–HEPES, 0.1 mM $MgSO_4$, 2–3 mg ml^{-1} vesicular protein, pH 7.5, to which 50 μM plus tracer ([^{75}Se]SeMet, 0.45 μCi ml^{-1}) were added. Data are expressed as the mean ± SEM for three experiments. ●, Deficient; ○, selenite; ▲, SeMet.

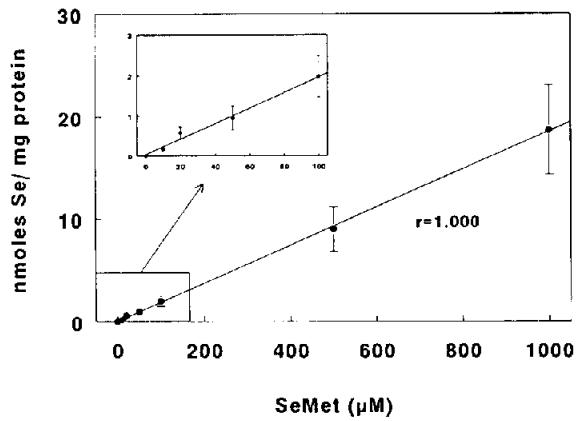

Figure 6. Selenium uptake from [^{75}Se]SeMet by BBMV over the concentration range 10–1000 μM after 30 s of incubation. BBMV were prepared from rats fed selenium-adequate diets. The incubation mixture at 37 °C contained 100 mM mannitol, 100 mM NaCl, 10 mM Tris–HEPES, 0.1 mM $MgSO_4$, 2–3 mg ml^{-1} vesicular protein, pH 7.5. Data are expressed as the mean ± SEM for three experiments.

diets were combined because no differences were observed for either the time course or concentration range measurements. The relationship between increasing selenium concentration and uptake is linear.

The uptake of ^{75}Se as selenite under Na^+-equilibrated conditions from rats fed selenium-deficient and -supplemented diets is shown in Figure 7. Although the uptake values appear slightly higher for the animals fed the SeMet supplemented diet, there were no statistically significant differences at any point during a 60 min time period among the diets. Uptake at equilibrium was in the range of 16.5–18.9 nmol mg^{-1} protein. There tended to be fairly large variations among replicate experiments performed using different preparations of BBMV. However, the

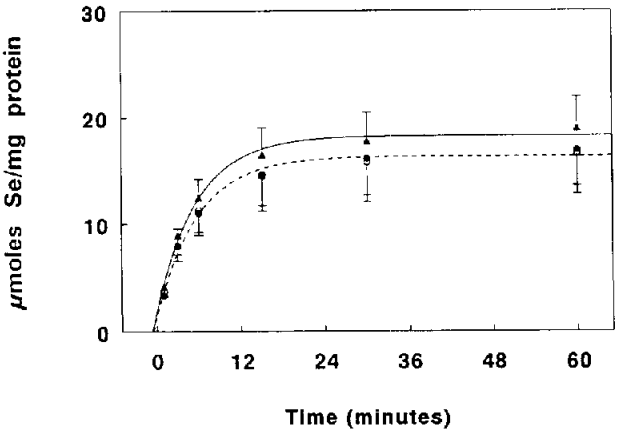

Figure 7. Selenium uptake from [^{75}Se]selenite by rat BBMV prepared from animals fed selenium-deficient and diets supplemented with either selenite or SeMet. The incubation mixture at 37 °C contained 100 mM mannitol, 100 mM NaCl, 10 mM Tris–HEPES, 0.1 mM $MgSO_4$, 0.25 mg ml^{-1} vesicular protein, pH 7.5, to which 50 μM selenite plus tracer ([^{75}Se]selenite, 0.45 μCi ml^{-1}) were added. Data are expressed as the mean ± SEM for seven experiments. Note that the y-axis is given as μmol Se μg^{-1} protein. ●, Deficient; ○, selenite; ▲, SeMet.

pattern of uptake over time was highly consistent and duplicate measurements were nearly identical. The control filters contained very little radioselenium when selenite was the form under study in contrast to SeMet where ^{75}Se binding by filters was high. Equilibrium uptake of selenite was approximately 50- and 8-fold higher than that for selenate and SeMet, respectively.

When ^{75}Se uptake was determined from [^{75}Se]selenite at concentrations ranging from 10 to 1000 μM (Figure 8) a curvilinear relationship was seen. The uptake measurements were terminated after 1 min. The uptake data was analyzed using Marquardt's Algorithm as described by

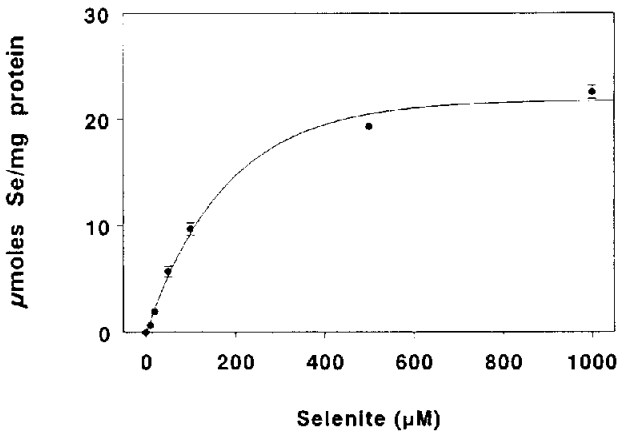

Figure 8. Selenium uptake from [^{75}Se]selenite by BBMV over the concentration range 10–1000 μM after 1 min of incubation. BBMV were prepared from rats fed selenium-adequate diets. The incubation mixture at 37 °C contained 100 mM mannitol, 100 mM NaCl, 10 mM Tris–HEPES, 0.1 mM $MgSO_4$, 0.25 mg ml^{-1} vesicular protein, pH 7.5. Data are expressed as the mean ± SEM for four experiments. Note that the y-axis is given as μmol Se μg^{-1} protein.

Duggleby (1984). The maximal rate of uptake, V_{max}, was 28.4 μmol min mg^{-1} vesicular protein and the K_t was 0.24 mM. There was no effect of a 100 mM Na^+ gradient on selenite uptake (data not shown).

To examine the binding of each selenocompound to the membrane, BBMV were prepared from rats fed a commercial rat diet. Uptake was determined after 30 min incubation in media of increasing osmolarity. Binding was determined for all three substrates on aliquots of four membrane preparations. Figure 9 shows the uptake as a function of the reciprocal of the medium osmolarity. Selenate (A) binds in the smallest quantities to the BBMV followed by SeMet (B) with the greatest amount of binding with selenite (C). Extrapolation to negligible intravesicular space reveals 6.5 nmol mg^{-1} protein of selenite bound, 2.4 nmol mg^{-1} for SeMet and 174 pmol mg^{-1} for selenate. Hence, selenite binding exceeds selenate by 37-fold and SeMet binding was 14-fold higher than selenate binding.

Discussion

The different diets had a significant influence on the yield of mucosal tissue which was used for the preparation of BBMV. As far as we know this is the first report on this observation. There is no explanation for a greater amount of mucosal tissue from the selenium-deficient rats as compared with the supplemented groups, but this could be due to several possibilities. First, selenium deficiency could produce ultrastructural changes resulting in fluid accumulation in the intestinal epithelium because of oxidative damage. Similar yields of homogenate protein from rats fed the three diets lends support to this suggestion. Second, it may have been more difficult to separate the mucosal cell layer from the underlying muscle as effectively in the deficient rats resulting in higher contamination by muscle tissue. Third, there may have been different proportions of various cell types due to dietary changes.

The significantly greater distribution of homogenate protein into the brush border fraction from selenite-fed as compared with SeMet-supplemented animals is intriguing. Selenite is known to readily form selenotrisulfides with tissue protein sulfhydryl groups and may be able to cross-link proteins in close proximity (Ganther & Corcoran 1969, Vendeland *et al.* 1992b). The specific activity of the brush border marker, sucrase, was higher for the SeMet-fed rats as compared with the selenite-fed animals, but the difference was not statistically significant.

The Na^+-dependent facilitated diffusion and active transport of glucose across the luminal membrane of the enterocyte has been well documented. Glucose uptake by BBMV from animals fed both selenium-deficient and -supplemented diets was very similar to results previously reported by others using BBMV from rats presumably fed standard commercial diets (Hopfer *et al.* 1973, Kessler & Toggenburger 1979).

The uptake characteristics of BBMV for selenite, SeMet and selenate described by the present results do not provide evidence for a specific transport system for these

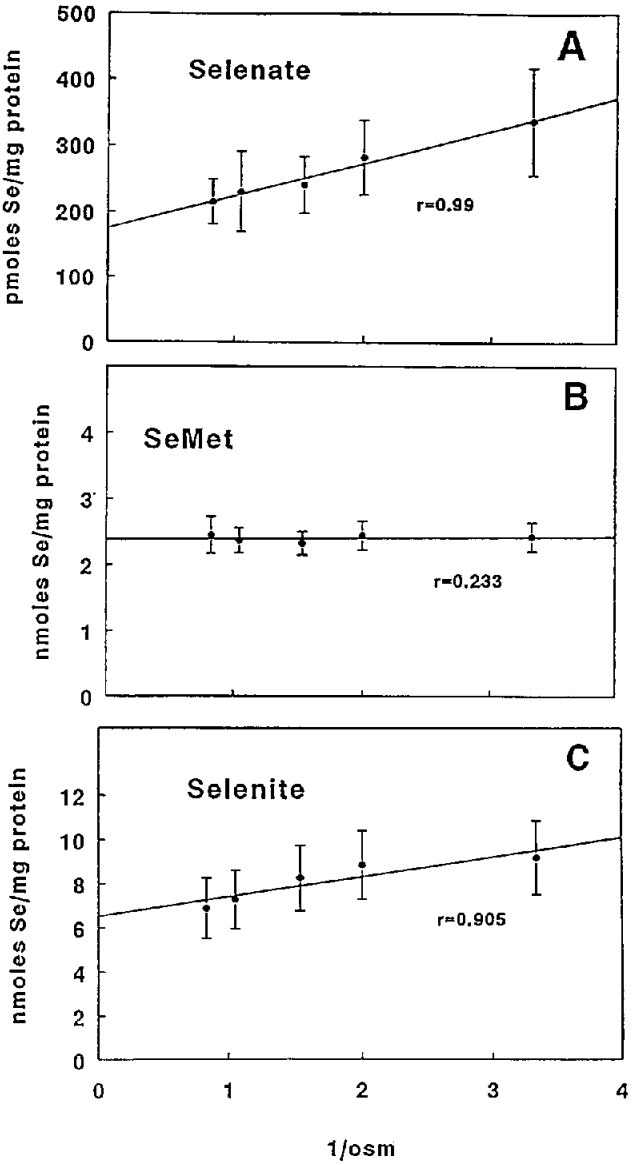

Figure 9. Binding of selenium from selenate (A), SeMet (B) and selenite (C) by BBMV as a function of the reciprocal of the osmolarity of the incubation meduim. Binding was determined in incubation mixtures at 37 °C containing 100 mM NaCl, 10 mM Tris–HEPES, 0.1 mM $MgSO_4$, pH 7.5. Various concentrations of mannitol ranging from 100 to 1000 mM, to which 50 μM plus tracer ([^{75}Se]selenate, [^{75}Se]SeMet or [^{75}Se]selenite) at 0.45 μCi ml^{-1} were added. Vesicular protein was present at 2–3 mg/ml for the binding assay using either selenate or SeMet and 0.25 mg ml^{-1} when using selenite. Data are expressed as the mean ± SEM (as indicated by bars) for four replicates.

forms of selenium localized on the luminal cell surface. Neither selenium status nor the form of dietary selenium supplementation altered transport characteristics of BBMV for selenate, selenite or SeMet.

Isolated BBMV have a tremendous capacity to take up selenium from selenite and SeMet which is several orders of magnitude above physiologic needs and normal intraluminal concentrations. Dietary selenium at 0.2 mg kg^{-1} translates to a concentration of approximately 0.5–1.0 μM

in the stomach and intestines. Dietary needs for zinc are approximately 100-fold higher than for selenium. However, the kinetic parameters derived for selenite uptake are similar in magnitude to those obtained in studies with zinc uptake into BBMV and are only slightly less than uptake data with calcium (Miller & Bronner 1981). The zinc transport system in normal rat BBMV manifested a V_{max} of 5.4 nmol min mg^{-1} protein and a K_t of 0.38 mM (Menard & Cousins 1983). BBMV from rat duodenum incubated in media ranging from 1 to 5 mM Ca exhibited a K_t of 1.1 mM (Miller & Bronner 1981). Although saturation data generally suggest the presence of a mediated transport system, it is also possible for diffusional systems to display saturation kinetics (Kessler & Toggenburger 1979) particularly at the relatively high concentrations of selenite used in these studies.

The uptake of selenium from selenite has generally been thought to occur by diffusion (McConnell & Cho 1965, Wolffram *et al.* 1985). Selenite may diffuse across the microvillar membrane and subsequently bind to sites on the internal membrane surface. BBMV from several species bind large quantities of selenite-derived selenium (Wolffram *et al.* 1986) and this binding has been shown to be primarily on the intravesicular surface (Mykkanen & Wasserman 1989). McConnell & Cho (1965) observed extensive binding of selenite-derived ^{75}Se to mucosal tissue proteins during absorption studies with everted gut sacs. The unusually high uptake of selenite raises serious questions about its use in physiologic experiments.

Selenate accumulated to a much smaller extent than either selenite or SeMet during incubation with BBMV. Equilibrium uptakes were similar in magnitude to results obtained previously for BBMV from rat ileum and pig jejunum (Wolffram *et al.* 1986). Studies on the uptake of selenate both *in vivo* (Wolffram *et al.* 1985) and *in vitro* (Arduser *et al.* 1985, 1986, Wolffram *et al.* 1986) have strongly supported a common transport system for selenate and sulfate (Lucke *et al.* 1981, Smith *et al.* 1981) located in the distal small intestine. These anions are transported by a Na^+ coupled electroneutral process across the brush border membrane of the ileum (Lucke *et al.* 1981, Arduser *et al.* 1985, 1986, Wolffram *et al.* 1986) and extruded from the enterocyte via a carrier-mediated exchange involving chloride and other anions (Langridge-Smith & Field 1981, Smith *et al.* 1981). The present results for selenate support the hypothesis that it crosses the microvillar membrane via a transport system designed primarily to transfer a substrate with a K_t far in excess of normal intraluminal concentrations of selenate. The lack of saturation over the range of selenate concentrations (10–1000 μM) is likely due to uptake by a carrier with a K_t about 1 mM and does not support a diffusion process. Comparison of selenite and selenate uptake by BBMV suggest that the latter compound might be preferable as a selenium source in physiologic experiments.

Similarly, the linear relationship of uptake as a function of increasing SeMet concentration is consistent with a shared transport system for a substrate having a K_t in the millimolar range. A common transport mechanism for SeMet and methionine was first suggested by McConnell & Cho (1967) using everted gut sacs from hamster small intestine. Recent work has provided strong evidence that SeMet entry into the enterocyte occurs via the electrogenic Na^+-dependent neutral amino acid transport system. Furthermore, the uptake of SeMet is kinetically indistinguishable from that of methionine (Wolffram *et al.* 1989).

In the absence of binding, the uptake at equilibrium depends only upon the selenium concentration and the internal volume of the vesicle preparation. Both the uptake and binding measurements indicate marked differences in the binding of the three selenocompounds to BBMV. The uptake of SeMet represents binding whereas the uptakes of selenite and selenate may include both binding and transport into the vesicle. SeMet binding to the BBMV far exceeded that of methionine using pig jejunal BBMVs (Wolffram *et al.* 1989). Possible binding sites for SeMet in addition to transport proteins have not been identified. In ligated loop studies, SeMet was retained by the intestinal tissue to the greatest degree although it was most efficiently removed from the lumen of the ligated segment. Zinc binding to BBMV at a medium concentration of 200 μM resulted in an estimate of approximately 1.0 nmol Zn bound per milligram protein at infinite osmolarity (Menard & Cousins 1983). Uptake of ferrous iron in uptake media containing 1.79 μM iron resulted in binding of 1.5 nmol mg^{-1} protein (Muir *et al.* 1984). At a medium concentration of 0.48 mM Ca, approximately 1.8 nmol mg^{-1} of the divalent cation was bound to the BBMV (Miller & Bronner, 1981). Considering the relative dietary requirements and likely intraluminal concentrations of these minerals as compared with the same parameters for selenium, the binding of selenium as selenite (6.5 nmol mg^{-1} protein) and SeMet (2.4 nmol mg^{-1} protein) seem to be tremendously excessive and unrelated to the normal process of trace mineral absorption. Selenate did not accumulate in BBMV in abnormally large quantities. These trends in binding closely parallel retention in intestinal tissue of these compounds during *in vivo* absorption experiments (Vendeland *et al.* 1992a). The question remains of any unknown effects of some forms of selenium and their interaction with BBMV and other components of the intestinal mucosa.

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