Selenium supplementation of infant formula: uptake and retention of various forms of selenium in suckling rats

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Formula-fed infants often have lower serum selenium levels than breast-fed infants. Although no deleterious effects have been correlated to this finding, supplementation of formula with selenium is considered. In this study, we investigated the uptake and retention by suckling rat pups of ^{75}Se from selenite, selenate, and selenomethionine added to infant formula. The molecular distribution of ^{75}Se in liver, kidney, intestine, and plasma was followed by gel-filtration chromatography on Superose 12. ^{75}Se-uptake was most rapid from selenomethionine (70% at 1 hr), followed by selenate (51%) and selenite (29%). This difference was explained by a higher retention of ^{75}Se in the stomach and small intestinal wall of pups given selenite supplement. Plasma distribution of ^{75}Se as studied by gel filtration was also different, with a higher proportion of ^{75}Se from selenomethionine being protein-bound than from selenite or selenate. Similarly, a larger proportion of ^{75}Se from selenomethionine became protein-bound in the liver than from selenite or selenate. In conclusion, although whole body retention after 24–48 hr was similar, the metabolic fate of selenium varies considerably with the form of selenium added to formula. Further studies are needed to study the long-term consequences of selenium accumulated in different body compartments.

Keywords: selenium; selenium supplementation; selenium retention; infant formula

Introduction

Infants fed cow's milk-based infant formula have been reported to have lower serum selenium concentrations than breast-fed infants,[1–3] and plasma glutathione peroxidase levels may also be reduced.[3] The reason for these observations is the lower amount of selenium provided by bovine milk or soy protein sources, on which the formulas are based, than from human milk obtained in most areas.[1–10] Although no clinical signs of selenium deficiency have been demonstrated in formula-fed infants, the Committee on Nutrition of the American Academy of Pediatrics has discussed the possibility of supplementing formulas with selenium to a level similar to that found in human milk. The form of selenium to use however, is still a subject of controversy. A number of studies have explored the effects of selenium supplementation in human adults,[11–13] employing either inorganic selenium in the form of selenite or selenate, or organic selenium in the form of selenomethionine (either directly as the seleno-amino acid or in the form of yeast). Fewer studies have explored the effects of increased selenium intake in infants. One study explored the effects of supplementing the maternal diet in Finland, a selenium-poor country, with different forms of selenium to increase breast milk selenium.[14] The mean milk selenium concentration was 7 μg/L for unsupplemented women, 11 μg/L for selenite supplemented women, and 14 μg/L for women supplemented with selenium in the form of yeast (primarily selenomethionine). Selenium intakes of the infants were 7.7, 8.9, and 11.5 μg/day, respectively. A second study evaluated the effect of selenium addition to formula in the form of sodium selenite. The serum selenium concentration was significantly higher in the group receiving fortified formula than in the group receiving unsupplemented formula.[2] Be-

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cause of potential early development of hypersensitivity, yeast is not a likely vehicle of fortification to be used for infants; the relevant choices would be selenite, selenate, or selenomethionine.

Several studies have explored the absorption of selenium in experimental animals. One study in suckling rats compared the absorption of selenium from selenite and selenomethionine, but their efficacy was only compared at 3 hr after intubation.[15] It has been shown recently that the efficacy and uptake kinetics for selenite and selenate are quite different, with the latter form being absorbed more rapidly,[13,16] and also shown that the kinetics of selenium uptake and retention from selenomethionine are considerably different from the two other selenium sources.[17,18] In addition, there has been some controversy with regard to whether selenite maintains its oxidation state or is actually reduced to elemental selenium in the presence of high levels of ascorbic acid.[12] Consequently it is essential to study the retention of selenium in different tissues and how it varies with time. The present study explores the effect of selenium form on tissue selenium distribution over time. Such knowledge is needed before strategies can be developed for supplementing infant formulas with selenium.

Materials and methods

Suckling Sprague-Dawley rat pups in litters of 10 (number of litters = 9) were obtained from a commercial vendor (Simonsen Laboratories, Gilroy, CA USA). At 14 days of age, animals were fasted for 6 hr prior to intubation with labeled formula. Within each litter, for each time point, 3–4 pups received infant formula (SMA, Wyeth Laboratories, Philadelphia, PA, USA) with either sodium salts of ^{75}Se-selenite, ^{75}Se-selenate, or ^{75}Se-selenomethionine (specific activity: 10.75 mCi/mg Se, radiochemical purity 96.1%; Amersham, Arlington Heights, IL USA) added as an extrinsic tag. The analyzed initial level of selenium in the formula was 8 μg/L. Cold selenium was added in the same form as the radiotracer at a level of 10 μg selenium/L. This level is in the same range as that of human milk and also commensurate with the proposed level of supplementation. The label was allowed to equilibrate for 24 hr at 4° C prior to intubation. Each animal received 1 μCi of ^{75}Se in 0.5 mL formula. Following another 3 hr fast post-dosing, pups were returned to their dams (except for those killed at earlier time points). Pups were killed at 1, 3, 8, 24, and 48 hours after intubation. A minimum of six pups per supplement and time point were used (6 × 3 × 5). Blood was drawn by cardiac puncture using heparinized syringes and counted; plasma was separated and immediately frozen following centrifugation. The small intestine was perfused with ice-cold saline (5 mL) and divided into three segments, approximately corresponding to duodenum, jejunum, and ileum. Perfusate and intestinal segments were also frozen and then counted separately. Liver, spleen, kidney, cecum-colon, and carcass were collected, frozen on dry ice, and counted in a gamma well-counter.

Liver, kidney, and small intestinal segments were homogenized (Tekmar homogenizer, 20 sec., Tekmar Co., Cincinnati, OH, USA) in saline (25% tissue wt/vol) and centrifuged at 10,000 rpm for 15 min. Supernatants and plasma were then fractionated by fast protein liquid chromatography (FPLC, Pharmacia, Piscataway, NJ USA) using a gel filtration column (Superose 12, Pharmacia) in 0.1 M ethanolamine buffer at pH 8.6. Eluted fractions were monitored for proteins at 280 nm and counted for ^{75}Se. All runs were done in duplicate; representative chromatograms are shown in figures (see Results).

The experimental protocol had been approved by the Animal Use Committee and by Environmental Health and Safety at the University of California at Davis campus. Sta-

Table 1 Selenium absorption/retention from various supplements ($\bar{X}$ ± SEM)

(n)		Stomach	Intestine 1	Intestine 2	Intestine 3	Cecum-colon
						(% of dose
SMA + Selenate						
1 hr	(5)	20.6 ± 4.1	15.5 ± 0.9^{a}	2.5 ± 0.4^{a}	1.2 ± 0.2	0.9 ± 0.1^{a}
3 hrs	(7)	1.7 ± 0.3^{a}	12.0 ± 1.0^{a}	4.9 ± 0.5^{a}	2.7 ± 0.2	1.6 ± 0.1
8 hrs	(6)	1.2 ± 0.1^{a}	8.0 ± 1.2^{a}	3.0 ± 0.4	3.0 ± 0.4^{a}	1.7 ± 0.2
24 hrs	(6)	1.6 ± 0.4	6.3 ± 0.8^{a}	2.5 ± 0.3^{a}	2.4 ± 0.1^{a,b}	2.2 ± 0.2^{a}
48 hrs	(7)	1.2 ± 0.4^{a}	4.7 ± 0.4^{a}	1.6 ± 0.1^{a}	2.5 ± 0.2^{a}	2.1 ± 0.2^{a}
SMA + Selenite						
1 hr	(5)	20.8 ± 0.8	20.6 ± 1.4^{b}	22.8 ± 1.6^{b}	2.2 ± 0.5	0.7 ± 0.1^{a}
3 hrs	(6)	7.5 ± 1.1^{b}	11.9 ± 0.8^{a}	14.5 ± 1.1^{b}	4.9 ± 1.1	1.8 ± 0.4
8 hrs	(6)	2.4 ± 0.5^{b}	3.0 ± 1.2^{b}	4.0 ± 2.1	2.2 ± 0.9^{a,b}	1.2 ± 0.3
24 hrs	(5)	2.2 ± 0.3	5.0 ± 0.8^{a,b}	5.2 ± 1.1^{b}	3.1 ± 0.5^{a}	2.7 ± 0.2^{a,b}
48 hrs	(6)	1.9 ± 0.1^{b}	4.9 ± 0.2^{a}	3.5 ± 0.3^{b}	2.9 ± 0.2^{a}	2.8 ± 0.2^{b}
SMA + Selenomethionine						
1 hr	(5)	13.6 ± 2.1	4.8 ± 0.9^{c}	1.9 ± 0.2^{a}	2.4 ± 1.0	1.3 ± 0.1^{b}
3 hrs	(7)	2.3 ± 0.3^{a}	4.5 ± 0.6^{b}	2.3 ± 0.2^{c}	2.8 ± 0.9	2.0 ± 0.3
8 hrs	(6)	1.4 ± 0.2^{a}	2.2 ± 0.5^{b}	1.2 ± 0.2	1.1 ± 0.1^{b}	1.4 ± 0.4
24 hrs	(6)	1.5 ± 0.2	3.2 ± 0.2^{b}	1.4 ± 0.2^{a}	1.8 ± 0.1^{b}	4.3 ± 1.0^{b}
48 hrs	(6)	1.2 ± 0.1^{a}	2.6 ± 0.2^{b}	1.4 ± 0.1^{a}	1.5 ± 0.1^{b}	2.4 ± 0.2^{a,b}

*(Liver + kidney + blood + carcass).
Different superscripts for each diet at each time point denote significant differences ($P < 0.05$) by ANOVA.

tistical evaluations were performed at each time point by analysis of variance and using Student's *t* test to detect differences among means.

Results

Selenium uptake into the body (whole body retention expressed as liver + kidney + blood + carcass) was most rapid when animals received selenomethionine, followed by selenate, and slowest from selenite *(Table 1)*. After 1 hour, 70% of the selenium dose given had been absorbed from selenomethionine, while only 29% of selenium had been absorbed from selenite, and selenate was intermediate (51%). At 3 hours, uptake of selenium from selenite was still lower than from the other forms of selenium given. This difference in uptake into the body is explained by a higher proportion of selenium found in the stomach and the perfused intestine when selenite was given as compared to selenate and selenomethionine *(Figure 1A and B)*. Initial uptake of ^{75}Se into blood compartments was also different for the three forms of selenium. At 1 hr, a higher proportion of blood ^{75}Se from selenomethionine was found in plasma (81%) than from selenate (73%) and selenite (68%) *(Figure 2)*. In addition, the distribution of ^{75}Se in plasma was different; for selenate, a large proportion (76%) of ^{75}Se was found to be of low molecular weight (LMW), while the largest proportion of ^{75}Se from selenomethionine (76%) and selenite (85%) was protein-bound [high molecular plus medium molecular weight (HMW+MMW)] *(Figure 3; Table 2)*. By 3 hr, there were no pronounced differences, either in RBC-plasma distribution or in molecular localization in plasma (as judged by gel filtration).

Eight hours after dosing, there were no differences between selenite and selenate with regard to selenium retention or tissue distribution, while total retention from selenomethionine was higher than that for inorganic forms of selenium *(Table 1)*. In addition, selenium retention in the small intestine was significantly lower from selenomethionine than from selenate and selenite *(Figure 1)*.

At 24 and 48 hr post-dosing, selenium retention from selenomethionine was lower in liver, kidney, and blood and higher in the remainder of the body than that from selenite and selenate. Total body retention of selenium, however, was similar for all three forms of selenium.

Liver, kidney, and small intestine homogenates were separated by fast protein liquid chromatography (FPLC) using a gel filtration column (Superose 12) *(Figures 4–6; Table 2)*. At 1 hr post-intubation, a significantly higher proportion (71%) of ^{75}Se from selenomethionine was found in LMW form than from either selenite (46%) or selenate (38%) in both the liver and kidney. By 3 hr however, a much higher proportion (66%) of ^{75}Se from selenomethionine was bound to proteins of MMW in the liver than from selenite (21%) and selenate (23%). These differences were less pronounced at 24 hr, although the highest proportion of protein-bound ^{75}Se was found for selenomethionine. A higher proportion of ^{75}Se was found in the cytosolic fraction for selenate at 24 hr as compared with the other forms of selenium, suggesting preferential incorporation into this pool. In the kidney, a larger percentage of ^{75}Se was found bound to HMW proteins than in the liver, regardless of the form of ^{75}Se given. In the small intestine, distribution of newly absorbed ^{75}Se was quite different; at 1 hr post-intubation only a small fraction (4%) of ^{75}Se from selenomethionine was present in LMW form, while 23% and 31%, respectively, of ^{75}Se from selenite and selenate was found in this fraction. A high proportion (32%) of ^{75}Se from selenomethionine was found in LMW proteins, as compared with the

Intestinal perfusate	Liver	Kidney	Blood	Carcass	Whole body retention*
given)					
1.6 ± 0.1[a]	6.4 ± 1.2[a]	1.8 ± 0.1[a]	7.4 ± 0.5[a]	36.8 ± 2.0[a]	50.8 ± 2.7[a]
0.8 ± 0.1[a]	12.0 ± 0.2[a]	4.0 ± 0.2[a]	11.6 ± 0.5[a]	44.9 ± 1.5[a]	72.5 ± 2.0[a]
0.4 ± 0.1	11.1 ± 0.6[a]	5.5 ± 0.3[a]	11.1 ± 0.6[a]	44.0 ± 3.6[a]	71.7 ± 3.7[a]
0.4 ± 0.1	10.4 ± 0.4[a]	5.7 ± 0.7[a]	7.3 ± 0.5[a]	43.5 ± 1.0[a]	67.2 ± 1.0[a,b]
0.3 ± 0.0	11.3 ± 0.4[a]	5.5 ± 0.3	7.1 ± 0.1[a]	49.3 ± 1.5[a]	73.2 ± 1.7[a]
5.3 ± 0.6[b]	6.8 ± 0.6[a]	1.4 ± 0.2[a]	5.7 ± 0.6[b]	14.9 ± 1.0[b]	28.8 ± 2.2[b]
2.7 ± 0.1[b]	13.3 ± 0.5[b]	4.0 ± 0.1[a]	10.7 ± 0.6[a]	26.2 ± 3.0[b]	54.3 ± 3.0[b]
0.5 ± 0.1	10.9 ± 1.0[a]	6.8 ± 0.4[b]	11.6 ± 1.0[a]	46.2 ± 3.8[a]	75.6 ± 4.7[a]
0.5 ± 0.1	10.6 ± 0.6[a]	6.0 ± 0.5[a]	8.2 ± 0.4[a]	44.9 ± 3.8[a]	65.3 ± 1.4[a]
0.3 ± 0.0	9.8 ± 0.9[a,b]	6.2 ± 0.7	7.8 ± 0.2[b]	49.2 ± 0.4[a]	73.0 ± 0.6[a]
1.6 ± 0.5[a]	10.4 ± 0.4[b]	2.3 ± 0.2[b]	5.7 ± 0.3[b]	54.7 ± 3.6[c]	69.7 ± 1.6[c]
2.7 ± 0.9[b]	9.2 ± 0.3[c]	3.2 ± 0.1[b]	6.4 ± 0.1[b]	58.2 ± 2.5[c]	77.0 ± 2.4[c]
0.3 ± 0.1	8.2 ± 0.3[b]	4.3 ± 0.1[b]	6.1 ± 0.4[b]	66.7 ± 0.8[b]	85.3 ± 2.3[b]
0.4 ± 0.0	8.2 ± 0.2[b]	3.6 ± 0.1[b]	4.8 ± 0.1[b]	53.3 ± 1.8[b]	69.9 ± 1.7[b]
0.5 ± 0.2	8.7 ± 1.0[b]	4.7 ± 1.0	5.0 ± 0.1[c]	58.7 ± 2.0[b]	77.0 ± 2.0[b]

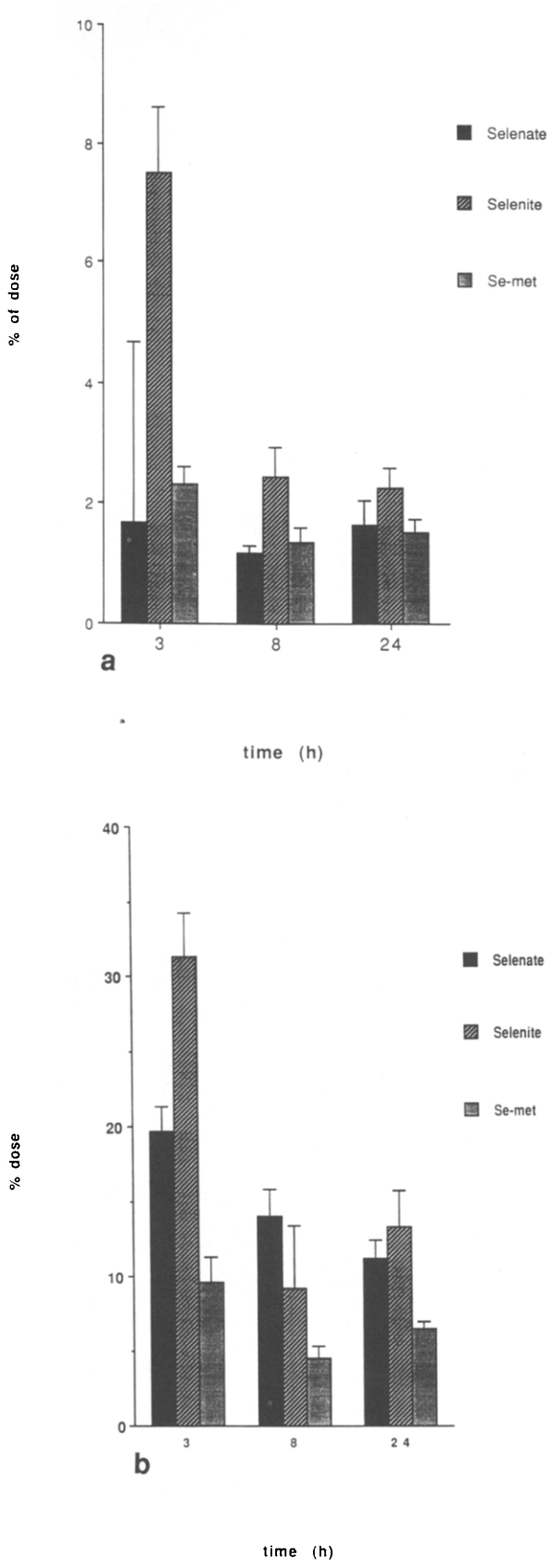

Figure 1 Retention in stomach and perfused small intestine of selenium given as selenate, selenite, or selenomethionine at various times post-intubation (n = 5–7/time point). a: stomach, b: small intestine.

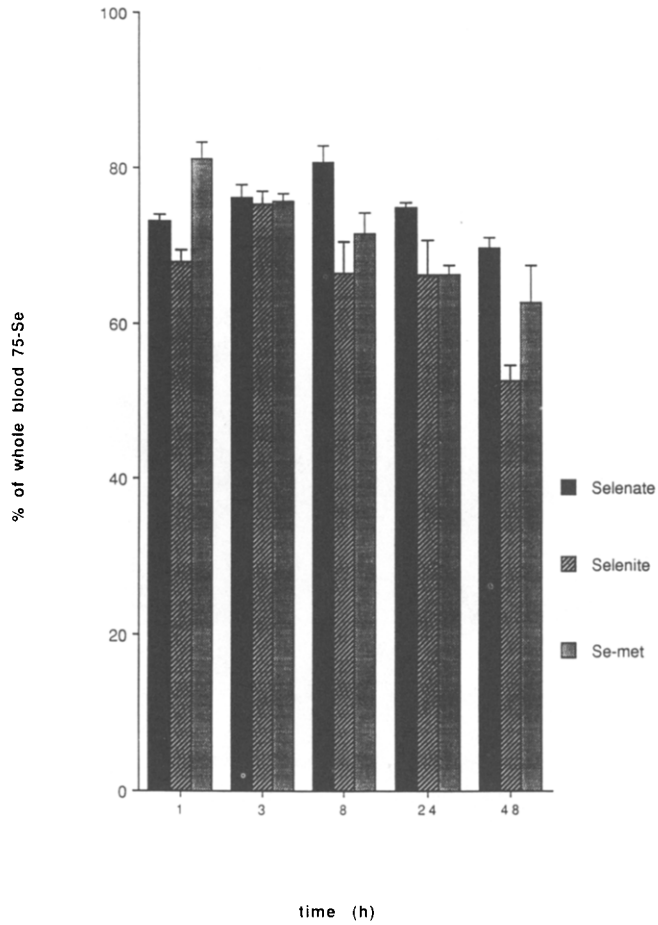

Figure 2 Appearance of absorbed selenium (^{75}Se) from selenate, selenite, or selenomethionine into plasma at various times post-intubation. Values are given as percent of whole blood ^{75}Se (n = 5–7/time point).

other two forms (2 and 9%, respectively). At 3 hr, most of the ^{75}Se from selenomethionine had left the small intestine; what remained was found largely (59%) in LMW form. For the other two forms of ^{75}Se, a higher proportion of ^{75}Se remained in the small intestine and was largely bound to proteins. Similar results were obtained at 24 hr post-intubation.

Discussion

It should be recognized that the purpose of this study was to follow the absorption and tissue distribution of the selenium supplemented to formula by using ^{75}Se-labeled supplements. It is questionable whether the radiolabel equilibrates with the native selenium in the milk formula; intrinsic labeling of cow milk may be necessary for studying this pool of selenium.[19,20] Before deciding on selenium supplementation of formula however, the handling of this additional pool should be studied in detail. Studies on tissue distribution and molecular localization will be impossible in human infants; we have therefore chosen to study this in the suckling rat pup model.

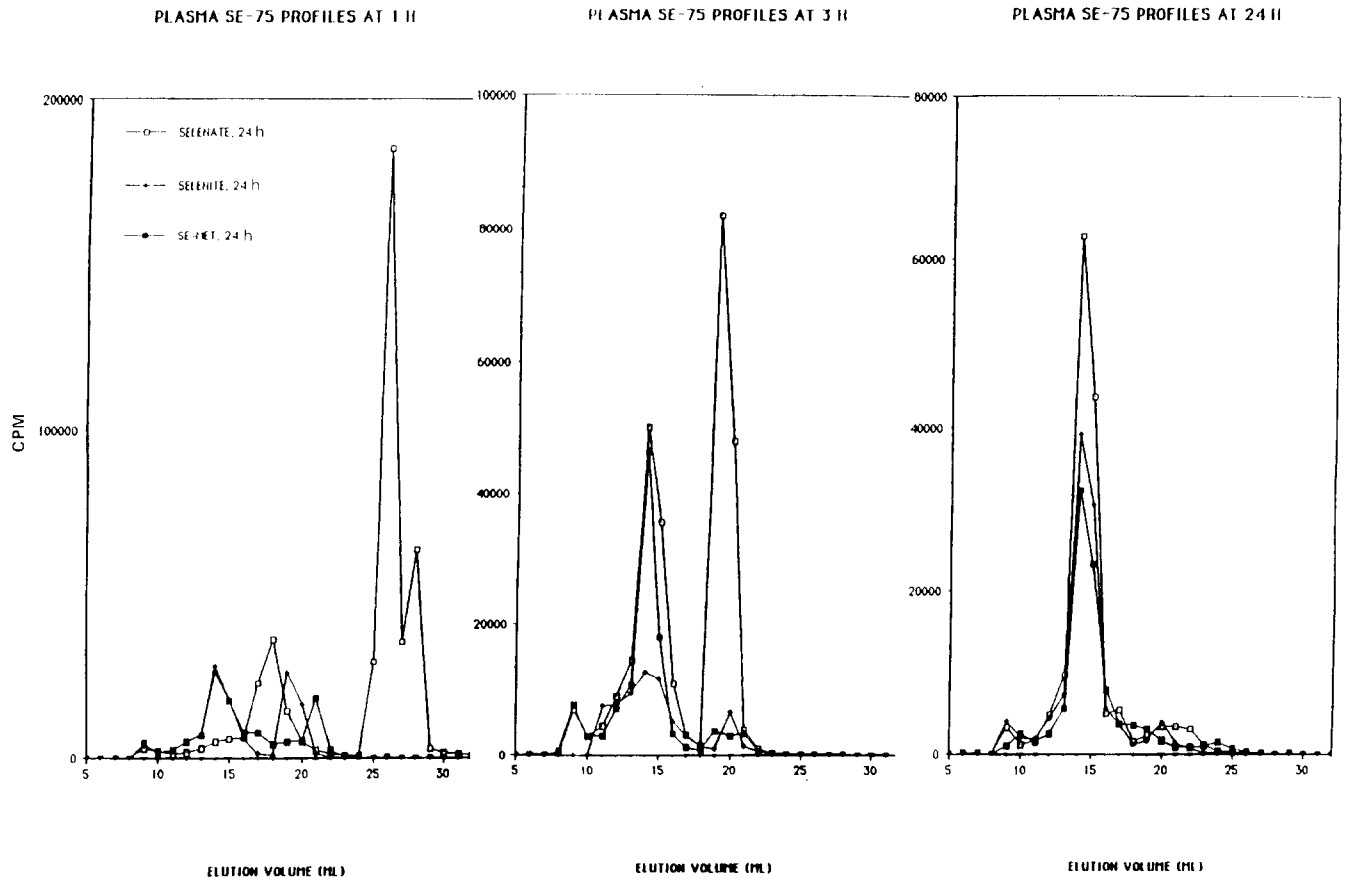

Figure 3 Distribution of absorbed ^{75}Se from selenate, selenite, and selenomethionine in plasma separated by FPLC gel filtration chromatography on Superose 12.

Absorption of selenium from selenomethionine was more rapid and higher than from selenite and selenate. This is consistent with other studies showing that circulating selenium levels are higher when selenomethionine is given as a supplement compared with selenite.[11] Although whole body retention at 24 and 48 hr was not significantly different among the groups in this study, only a single dose was given, and it is possible that with chronic dosing the higher uptake in the selenomethionine group would result in higher selenium status. It should also be noted that we used infant, suckling animals in this study. Virtually all previous studies have been done in older animals, and it is possible that there are no pronounced differences in whole body retention of selenium from different forms after 24 hr in infants, even if pronounced differences in tissue and molecular distribution may be found.

Tissue uptake and distribution of selenium was also found to vary with the selenium supplement used. Significantly higher proportions of selenite and selenate remained in the stomach and small intestine for a

Table 2 ^{75}Se distribution (%) among high, medium, and low molecular weight peaks as separated by Superose-12 FPLC column chromatography

Treatment	Time	Liver		
		HMW	MMW	LMW
SME + selenate	1 hr	0.3	59.3	38.4
SME + selenite	1 hr	0.4	52.2	45.8
SMA + selenomethionine	1 hr	0.3	26.8	71.1
SMA + selenate	3 hr	0.1	22.7	76.0
SMA + selenite	3 hr	4.3	21.0	68.5
SMA + selenomethionine	3 hr	0.2	65.6	33.2
SMA + selenate	24 hr	0.1	68.3	30.8
SMA + selenite	24 hr	0.5	56.0	42.5
SMA + selenomethionine	24 hr	0.2	77.0	21.9

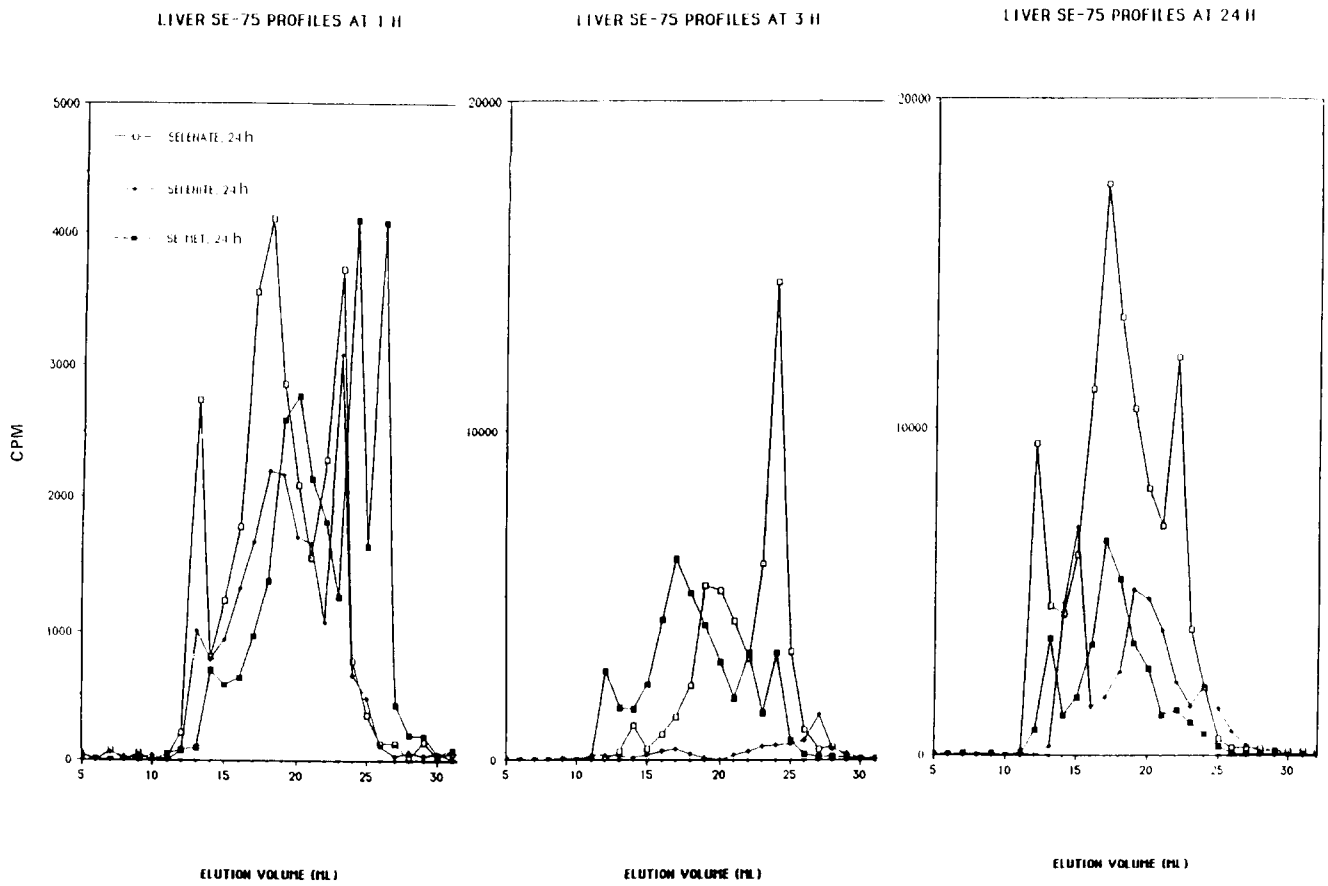

Figure 4 Distribution of absorbed ^{75}Se from selenate, selenite, and selenomethionine in liver separated by FPLC gel filtration chromatography on Superose 12.

longer time as compared with selenomethionine, suggesting different pathways of absorption. The more rapid clearance of selenomethionine found in our study is similar to what has been described for chickens using ligated intestinal loops.[17] Selenomethionine appears to be absorbed via a carrier-mediated process, which is similar to that used for methionine.[18] Of the two inorganic forms, selenate was more rapidly incorporated into the body than selenite. When comparing the intestinal uptake of these forms in a rat in vitro perfusion system, Wolffram et al.[16] found that selenate was more rapidly absorbed, most likely via a carrier-mediated process. These differences in absorption mechanisms are reflected in our results from FPLC gel filtration separations of mucosal homogenates.

Selenium from selenomethionine was more rapidly taken up into the carcass than when given as selenite or selenate. It has been shown previously that selen-

Kidney			Intestine			Plasma		
HMW	MMW	LMW	HMW	MMW	LMW	HMW	MMW	LMW
30.2	61.3	8.0	2.3	66.1	31.0	1.3	21.6	76.2
0.8	83.7	12.2	9.2	67.8	22.7	6.4	78.3	15.2
8.7	48.0	42.6	32.3	63.3	3.9	7.3	68.8	23.8
23.6	67.8	8.0	4.2	64.4	30.7	5.2	75.3	19.5
37.3	56.0	6.3	19.4	63.1	15.9	11.0	74.4	13.9
12.0	56.7	30.9	3.4	37.6	58.7	12.5	80.4	6.9
21.3	62.2	16.4	2.9	69.1	27.3	3.9	90.1	5.6
29.7	58.2	11.4	3.4	69.3	27.1	7.1	86.6	6.0
18.4	71.6	9.7	1.1	42.3	56.5	4.9	87.5	7.2

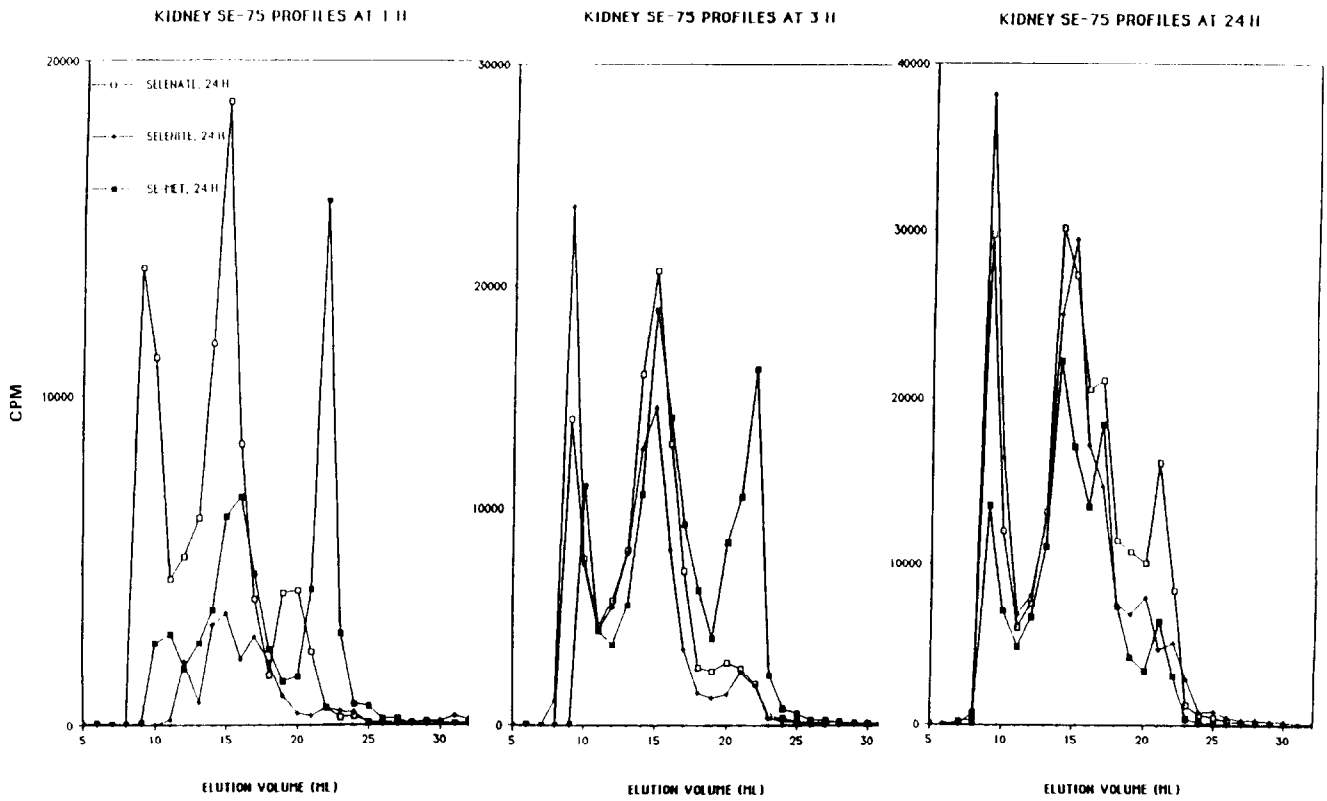

Figure 5 Distribution of absorbed ^{75}Se from selenate, selenite, and selenomethionine in kidney separated by FPLC gel filtration chromatography on Superose 12.

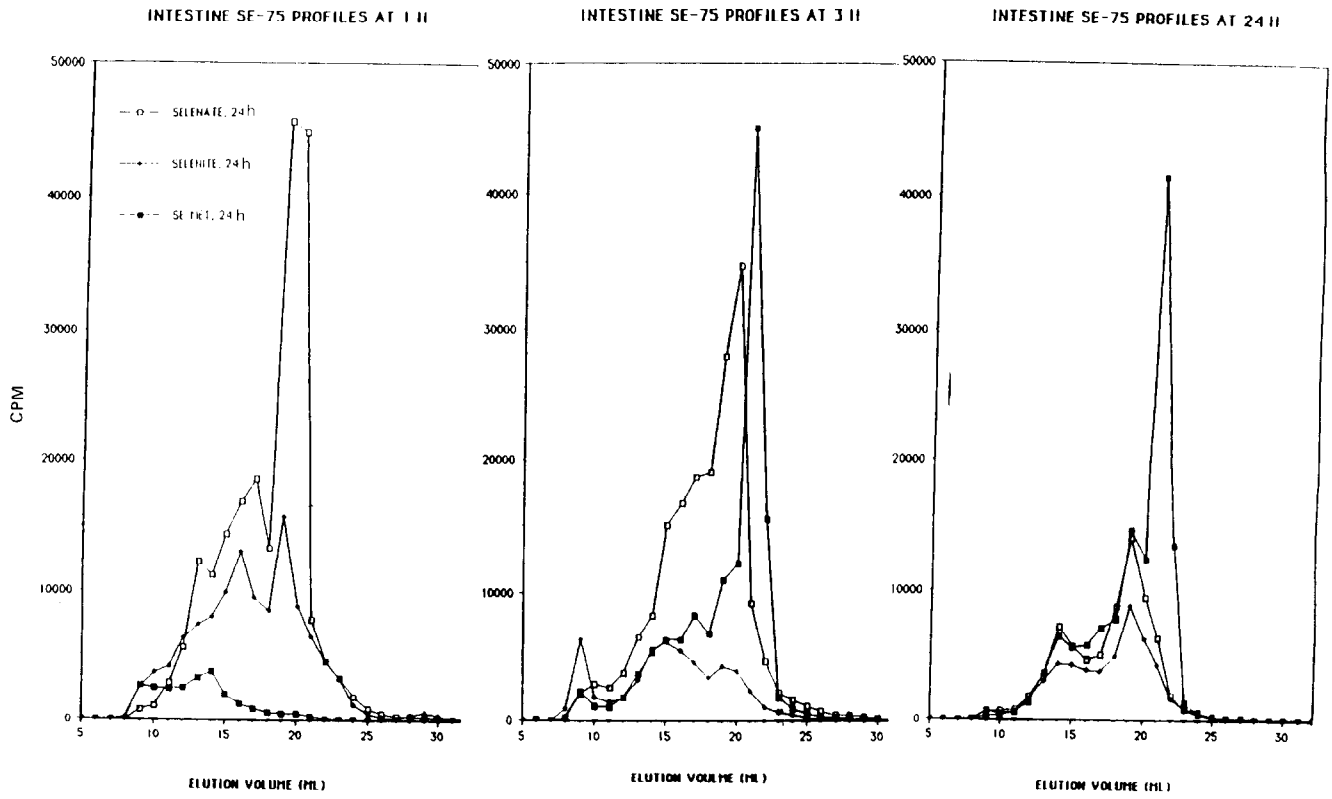

Figure 6 Distribution of absorbed ^{75}Se from selenate, selenite, and selenomethionine in the small intestine separated by FPLC gel filtration chromatography on Superose 12.

omethionine is more efficiently taken up into the body[21] and into muscle[22] than selenium from selenite. It is possible that the organic form of selenium is preferentially incorporated into newly synthesized soft tissue such as muscle. The higher uptake by the carcass (soft tissue) is also reflected by lower retention of selenium in the blood, suggesting that selenium in blood is a readily available pool of this element for various target tissues. However, once absorbed, selenite may be slightly better utilized for synthesis of glutathione peroxidase. This was shown in a recent study[23] in which these two forms of selenium were administered intraperitoneally. The inorganic forms of selenium are converted into selenocysteine and may be incorporated directly into glutathione peroxidase. In contrast, selenomethionine must be catabolized to free selenium before synthesis of selenocysteine can occur.[22] However, selenomethionine may be incorporated into methionine-containing proteins directly, because methionine t-RNA recognizes selenomethionine. Results of Thomson et al.[11] indicate that selenium-deficient adult humans receiving selenomethionine supplementation have higher concentrations of protein-associated selenium than individuals receiving inorganic selenium salts. However, glutathione peroxidase activities increased to a similar degree in both groups. The same group has recently shown that selenate is preferentially incorporated into glutathione peroxidase in both red blood cells and plasma as compared with selenomethionine.[24] Because glutathione peroxidase is a major selenium-requiring enzyme, supplementation with a form of selenium that is specifically incorporated into this enzyme may be advantageous. The possible cytotoxicity of selenomethionine should also be considered.[25]

The concentration of selenium in human milk is known to be highly variable. It appears, however, that the selenium level of infant formula may be significantly lower than that of human milk. As a consequence of a lower intake of selenium, formula-fed infants may have lower levels of blood selenium than do breast-fed infants.[1,2] A recent study by Litov et al.,[10] however, indicated similar selenium status in breast-fed infants and infants fed unsupplemented or selenium-supplemented cow's milk formula or soy formula, although their plasma selenium values were found to be higher than those reported in most other studies.[1,2] While lower levels of circulating selenium have been found in formula-fed infants, they have not yet been associated with any signs of selenium deficiency, but in analogy to other nutrients (such as vitamins), it has been suggested that the selenium status of formula-fed infants should be equivalent to that of breast-fed infants. It has recently been shown that breast-fed infants are in positive selenium balance, while formula-fed infants frequently have a negative selenium balance.[26] While this study does not directly answer the question of which form of selenium supplement should be used if such a decision is made, it emphasizes that different forms of selenium will be handled differently metabolically.

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