

Human selenite metabolism: a kinetic model

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PATTERSON, B. H., O. A. LEVANDER, K. HELZLSOUER, P. A. McADAM, S. A. LEWIS, P. R. TAYLOR, C. VEILLON, AND L. A. ZECH. *Human selenite metabolism: a kinetic model*. *Am. J. Physiol.* 257 (Regulatory Integrative Comp. Physiol. 26): R556-R567, 1989.—A model is developed to describe the kinetics of sodium selenite metabolism in humans, based on plasma, urine, and fecal samples obtained from six subjects over a 4-wk period after a single oral 200- μ g dose of the enriched stable isotope tracer ^{74}Se . The model describes absorption, distributed along the gastrointestinal tract, and enterohepatic recirculation. The model includes four kinetically distinct plasma components, a subsystem consisting of the liver and pancreas, and a slowly turning-over tissue pool. For the six subjects, the ranges of mean residence times for the four plasma components are, respectively, 0.2–1.1 h, 3–8 h, 9–42 h, and 200–285 h; for the hepatopancreatic subsystem 4–41 days; and for the tissue pool 115–285 days. Approximately 84% of the administered dose was absorbed, and after 12 days ~65% remained in the body. The model predicts that after 90 days ~35% of this Se would be retained, primarily in the tissues. Separating Se metabolism into several distinct kinetic components is a first step in identifying the efficacious, nutritious, and toxic forms of the element.

selenium; trace elements; compartmental modeling; pharmacokinetics; modeling; cancer; requirements

SELENIUM WAS SHOWN in 1957 to be an essential nutrient in animals (30). Its biochemical functions were unknown until 1973, when Se was identified as a component of glutathione peroxidase (29), an enzyme that protects against oxidative damage, presumably by catalyzing reduction of lipid hydroperoxides (12). Accumulated evidence suggests that Se is required by humans (20), and the National Research Council recommends as safe and adequate a daily intake for adults of 50–200 μ g (25). Severe Se deficiency has been associated with a cardiomyopathy among children in central China; conversely, very high doses are toxic to humans (7).

Se supplementation has been shown to inhibit tumorigenesis in several organ system models in animals. In 35 Se supplementation studies in rats, mice, and hamsters, Se produced an inhibitory response in 31, no response in 3, and an enhancement of tumorigenicity in 1 (14). The mechanism by which Se inhibits neoplastic transformation is not well understood.

The possibility that Se is a potential cancer-prevention agent in humans has been suggested by some ecological

and epidemiologic studies, although others have given equivocal results. In a review of studies conducted to investigate the hypothesis that Se status is related to cancer risk in human populations, Combs (6) concludes that they provide limited support for this hypothesis.

Because of its potential as a cancer-prevention agent, a greater understanding of Se metabolism in humans is necessary; little information is available in the literature on the integrative and regulatory metabolism of Se. A pharmacokinetic study utilizing a stable isotope tracer of sodium selenite, a candidate intervention agent, was undertaken to develop a kinetic model from which estimates of basic metabolic parameters for sodium selenite could be made.

METHODS

Subjects and protocol. Six subjects, three males and three females, from the Beltsville, MD, area were recruited into the study. The study protocol had been approved by the National Institutes of Health and United States Department of Agriculture Safety and Protocol Monitoring Committees. Subjects signed informed consent statements and were paid for participation in the study. They were required to be in general good health and could be taking neither Se supplements nor other medications. Female subjects could be neither pregnant nor lactating. Screening consisted of a complete medical history, physical examination, and laboratory tests (hematological, biochemical, and urinalysis profiles). Subject characteristics are given in Table 1.

Subjects were fed the same daily diet for 3 days before dose day (*day 0*) and 12 days afterward. All food was formulated and prepared at the Beltsville Human Nutrition Research Center. The diet furnished 87 μ g of dietary Se per day, 94 g of fat, 304 g of carbohydrates, and 98 g of protein. Deionized drinking water was provided, and caloric intake was supplemented with Se-free lemon-lime soft drinks. Subjects consumed a self-selected diet before and after the special diet days.

On *day 0*, subjects were given a single labeled oral dose of 200 μ g of enriched ^{74}Se (77.71% ^{74}Se) as selenite ($^{74}\text{SeO}_3$) in distilled deionized water. This stable isotope has a natural abundance of 0.88 mass atom percent (13). The enriched label was obtained as elemental Se from the Oak Ridge National Laboratory, Oak Ridge, TN. It was dissolved in a minimal amount of HNO_3 and diluted to volume, such that the concentration was 20 μ g $^{74}\text{Se}/$

TABLE 1. *Subject characteristics*

Characteristic	Subject No.					
	1	2	3	4	5	6
Age, yr	25	28	27	44	26	39
Sex	M	M	M	F	F	F
Weight, kg	80.6	65.6	83.3	60.0	58.0	54.7
Ideal weight,* kg	84.7	72.5	86.1	46.4	58.9	57.8
Ideal weight, %	95	91	97	129	99	95
Height, cm	186	176	187	154	168	167
Plasma vol,† liters	3.6	3.0	3.7	2.7	2.6	2.5
Hematocrit, %	44.1	43.0	43.5	40.7	42.6	38.4
Plasma Se,‡ ng/ml	133	133	119	118	136	122

* See Davidson (8); † estimated at $0.045 \times$ subject weight (in kg); ‡ average values of total plasma Se for study days (excluding dose day).

ml (i.e., 10 ml/dose). Subjects fasted for 12 h before and 3 h after dosing. Three subjects (1, 4, and 6) were given a daily dose of an additional 200 μ g of unenriched Se as selenite on days 3–5 and 9–11 as part of a separate investigation.

Sample collection. All materials used for collecting and measuring specimens were determined to be Se-free. On dose day, blood was drawn immediately before the dose was administered, at 30 min, 60 min, hourly for the next 7 h, daily for days 1–5 and 11, then weekly for 2–3 wk. After dose day, all samples were drawn while subjects were fasting. Samples were drawn into 12- or 25-ml disposable syringes containing acid citrate dextrose using either butterfly-infusion sets or disposable needles. They were separated in a refrigerated centrifuge at 2,000 g for 15 min, plasma was drawn off with a disposable pipette, and aliquots were placed into cryotubes (Thomas Scientific) and quick frozen within 45 min of collection. Samples were stored frozen at -20°C .

Two-hour urine collections were made for the first 8 h, then a single 4-h collection followed by a single 12-h collection. Twenty-four-hour collections were made for the next 11 days. Two-, 4-, and 12-h urine collections were stored in 32-oz multipurpose polystyrene containers (Miles Laboratories), and 24-h urine collections in 2-liter disposable boxes (Curtin Mathison Scientific) with plastic bag liners. Urine samples were measured, and 100-ml aliquots were placed in plastic bottles (Walter Sarstedt) and stored frozen at -20°C until analysis.

Daily fecal samples were collected for 12 days, beginning on dose day. Fecal samples were collected in pre-weighed 32-oz polystyrene containers (Miles Laboratories), refrigerated, and then returned to the laboratory, where they were homogenized in a colloid mill (Greerco model MV-6-3 Laboratory Micro-Mill, Sellers Process Equipment, King of Prussia, PA) using deionized water. Weighed aliquots were then lyophilized for 6 days, re-weighed, and stored frozen at -20°C until analysis.

Sample preparation and analysis. A known quantity of enriched ^{82}Se was added to each sample before digestion with HNO_3 , H_3PO_4 , and H_2O_2 . Undigested lipids were extracted with chloroform, and any selenate was reduced to selenite with HCl. The selenite reacted with 4-nitro-*o*-phenylenediamine (NPD) to form 5-nitropiazselenol (Se-NPD). The Se-NPD was then extracted into chloroform for subsequent analysis by combined gas chromatography-mass spectrometry (Finnigan model 4000,

Finnigan, Sunnyvale, CA). From isotope ratio measurements in the Se-NPD⁺ parent ion cluster, the amount of ^{74}Se tracer, unenriched (natural) Se, and total Se in the sample could be quantitated. Complete details of this procedure have been published (26).

Kinetic modeling. Using the techniques of compartmental analysis, we developed a kinetic model for human selenite metabolism to account simultaneously for the plasma, urine, and fecal data. Simulation of the compartmental models presented was carried out using the SAAM/CONSAM simulator (3) on a VAX-11/780 computer system (Digital Equipment). Several models were proposed to describe the data, until one was developed that provided an adequate fit to all the observed data. Initial estimates for the parameters were obtained from the literature or were based on physiological constraints. When the final model was developed, the parameters were adjusted individually for each subject using nonlinear least-squares techniques to obtain a best fit of the data, as judged both visually and by the sum of squared deviations of the model-calculated values from the observed data. Utilizing the final compartmental model and the set of parameters for each subject, we calculated mean residence times (the average amount of time the tracer spends in a compartment or compartments).

Plasma ^{74}Se concentrations, which were measured in nanograms per milliliter, were converted to fraction of dose per milliliter by dividing by the dose. Urine and fecal values, measured in micrograms, were divided by 200 (the size of the dose) to give fraction of dose per sample.

MODEL DEVELOPMENT AND RESULTS

The a priori model. We began with the a priori model (Fig. 1), which was based on the study design assumptions that Se is absorbed into and excreted from a single

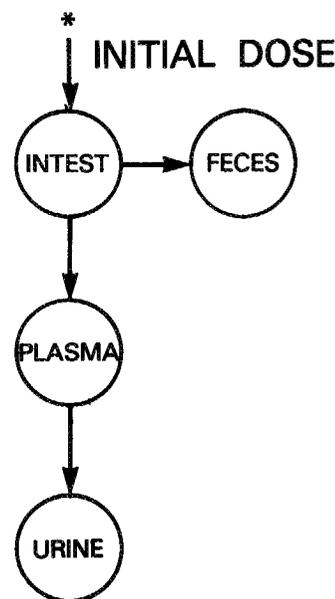


FIG. 1. A priori model for kinetics of selenite metabolism. Arrow with an asterisk indicates site of entry of oral tracer Se. Arrows between compartments represent pathways of fractional transport. This model has a single homogeneous plasma pool.

plasma pool. This model includes a single compartment representing the intestine (INTEST), which allows for a delay in absorption consistent with an oral dose. Absorption is modeled as occurring from this single homogeneous pool into a central plasma compartment, with removal into the urine. Compartments for the urine and the feces are required to take into account excreted label (Table 2).

Two inconsistencies are immediately apparent between this simple model and the observed data. About 35% of the administered dose was excreted during the first 12 days after dosing; of the ~65% that remained in the body, only ~9% was present in the plasma (by calculation). This model does not contain a compartment in which material can be sequestered. In addition, it does not explain the continued appearance of label in the feces throughout the study, seen in the positive slope of the cumulative fecal curve presented in Fig. 2. (We refer here only to the triangular data points; dashed lines represent the fit to the data of the final model, which is discussed later.) This suggests that there is enterohepatic recirculation of label, that is, that some label is returned to the intestine from the liver and pancreas via the bile and/or pancreatic juices.

Intermediate model. Our intermediate model, shown in Fig. 3, corrects the above deficiencies and better characterizes Se metabolism. In this model, the dose is introduced into a compartment from which no absorption occurs, perhaps the esophagus and the stomach, simulating a short delay before absorption begins. Absorption is modeled as being distributed along a chain of three compartments, which are assumed to have equal residence times, rather than as occurring from a single compartment. This configuration, which can be interpreted as representing the small intestine, better describes the physiology of absorption than did the a priori model and provides a somewhat improved fit for the first several hours of plasma data. Material that is not absorbed appears in the feces after a delay. This delay simulates time spent in a compartment that may represent the large intestine.

Material is absorbed into a single compartment, which is interpreted as representing the portal vein. From this compartment, some of the material moves directly into a second compartment (LIVER) from which it returns to the chain of compartments where absorption occurs. This might represent material that is absorbed from the gut into the portal circulation and then taken up by the liver on its initial pass through this organ before entering the general circulation (first-pass effect) and finally returned to the intestine via enterohepatic recirculation. The addition of this pathway permits a reasonable fit of

the positive slope of the cumulative fecal curve. The remaining ^{74}Se tracer moves from the compartment representing the portal vein into the plasma. This simulates material passing through the liver but not removed on the first pass through this organ. To help account for the material that remains in the body but is not present in the plasma, we introduced a slowly turning-over tissue compartment that exchanges with the plasma compartment.

This model, however, is inconsistent with the observed plasma and urine data, both of which suggest that, instead of a single plasma pool, there are multiple, kinetically distinct, plasma pools. A single pool might represent a single form of Se present in the plasma, and multiple pools might represent different Se compounds or metabolites. A single pool is consistent with a single peak in the plasma curve. In all study subjects, multiple peaks and troughs are apparent in the early plasma data (Fig. 4). The number and shape of these oscillations suggest that they represent distinct populations of Se and are not random noise in the data. The initial steep rise and subsequent slow increase in the cumulative urine curve, seen in all subjects (shown by circular data points for two subjects in Fig. 2), indicate that ^{74}Se was cleared more rapidly on dose day than on subsequent days, a phenomenon inconsistent with a single plasma pool from which label is cleared at a constant rate but consistent with multiple plasma pools with different clearance rates. In addition, this model is unable to account simultaneously for the quantity of label in the plasma and urine. When parameters are adjusted to fit the observed urine data (Fig. 5, top, dashed line), plasma levels predicted by the model (Fig. 5, bottom, dashed line) are one order of magnitude lower than those observed (Fig. 5, square data points). This discrepancy suggests that, in addition to the postulated single plasma pool, there is a very slowly turning-over plasma pool that contributes label to the long flat tail of the plasma curve. This second pool has a different, slower clearance rate into the urine than does the single plasma pool in the model and contributes only very small amounts to the urine.

Selenite model. A more complex model, the selenite model, is shown in Fig. 6, and associated parameters for the six subjects are given in Table 3. Label is introduced directly into the first compartment in a chain of compartments, G1-G3, from which absorption occurs. Material is absorbed into a rapidly turning-over pool, ENT, from which it leaves by two pathways; proportions following each are given in Table 3. The first pathway is represented by an arrow to the first plasma component P1 and a branch from this arrow, shown in dashed lines, to L/P. The second pathway is shown by the arrow passing through the compartment labeled HPL to a second plasma component P2. Compartment designations are discussed in the following paragraphs.

We speculate that ENT represents the intestinal cells, or enterocytes. The delay before the absorbed label appears in the plasma is modeled as occurring during passage through the gut and the intestinal cells, instead of preceding absorption as in the intermediate model. Because we were unable to determine exactly where the

TABLE 2. Cumulative fraction of excreted ^{74}Se (%dose) after 12 study days

	Subject No.						Means \pm SE
	1	2	3	4	5	6	
Urine	20.7	12.6	18.0	14.4	19.4	15.8	16.8 \pm 1.3
Feces	23.0	21.0	18.7	14.6	14.5	19.4	18.5 \pm 1.4
Total	43.7	33.6	36.7	29.0	33.9	35.2	35.4 \pm 2.0

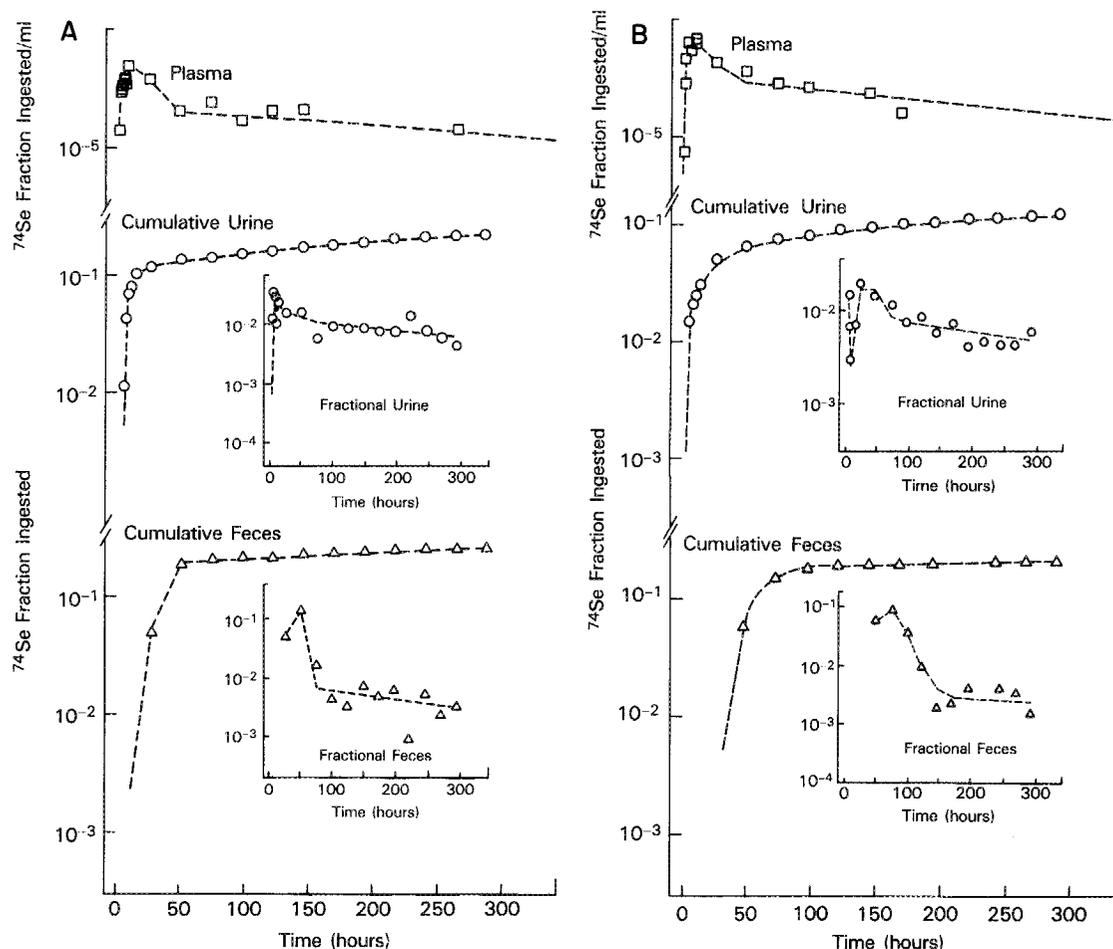


FIG. 2. Plasma, urine, and fecal tracer data for subjects 1 (A) and 2 (B) for 1st 300 h after dosing. Cumulative urine and feces data represent sum of fractional data shown in insets. Dashed lines indicate simultaneous fit of observed plasma, urine, and fecal data using selenite model. This is a compound figure: sometimes data alone are referenced and sometimes both data and fit to the data are referenced.

delay occurred, this model has no specific delay component. We further speculate that the first pathway from ENT represents material absorbed directly into the portal circulation. Label following this pathway appears in the peripheral circulation shortly after dosing and is represented by the plasma pool P1. Label in this pool can be seen as a "shoulder" on the early plasma curve for subjects 3 and 5 (Fig. 4: 30-min and 1-h data points). Material in P1 is rapidly removed from the plasma in all subjects. The rapid appearance and disappearance of label in this first plasma component, as predicted by the model, can be seen in Fig. 7, which shows the first 50 h of data for a single subject. With this subject, as with most of the others, the timing of the appearance in the plasma of a second component masks the rapid disappearance of the first (Fig. 4). The disappearance, however, is not totally masked for subject 5. This pool may represent material in the portal circulation passing through the liver before appearing in the plasma but not removed in this first pass. The model also allows for the possibility that some of the material in the portal circulation is removed by the liver before appearing in the plasma, as indicated in Fig. 6 by the dashed line to a pool, L/P, that represents the liver and pancreas. The

physiology of absorption suggests that this pathway exists, at least in nonfasting subjects. In fasting subjects, this pathway probably is insignificant and indeed may not exist. In this study, it improves the fit to the data for only one subject. This aspect of the model cannot be adequately tested without further studies designed to be sensitive to this issue.

The remainder of the absorbed dose follows a second pathway from the enterocyte, passing through the HPL compartment into a second plasma pool P2. As discussed below, HPL might represent a compartment in the hepatopancreatic subsystem or it might represent lymphatic flow from the enterocytes. The delay in the appearance of label in the plasma is a result of the time the label takes to move through HPL. Material in the second plasma component can be seen in several of the plasma curves as a slightly rounded "hump" adjacent to the shoulder between the 2-h and the 5- or 6-h data points (Fig. 4). This plasma pool is shown as a delay (Fig. 6) because of the length of time label spends in it, ~ 4.4 h on the average.

From the two plasma pools, P1 and P2, the tracer can be excreted in the urine or it can move into the compartment that represents the liver and pancreas. After a

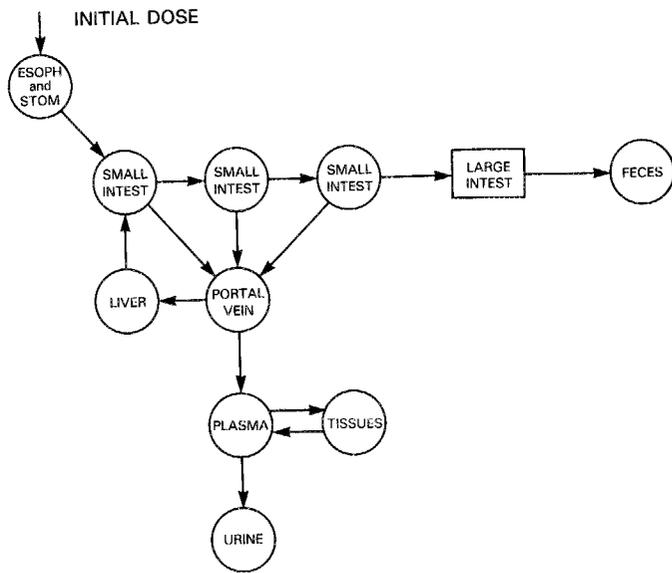


FIG. 3. Intermediate model for kinetics of selenite metabolism. Arrow with an asterisk indicates site of entry of oral tracer Se. Arrows between compartments represent pathways of fractional transport. Compartment depicted as a rectangle represents a delay. ESOPH, esophagus; STOM, stomach; INTEST, intestine. This model has enterohepatic recirculation and a single homogeneous plasma pool that exchanges with a tissue pool.

delay of ~4–6 h, material leaves the liver/pancreas by two pathways. The first is into a compartment labeled "bile," which may include liver bile and pancreatic juices. This pathway returns material to the gut, simulating enterohepatic recirculation. The second pathway leads to a third plasma pool P3. Material following this pathway at ~8 h in *subjects 1, 3, and 6* (Fig. 4). The rise is more gradual in *subjects 2 and 5*, and probably occurred after 8 h in *subject 4*, so it cannot be seen in the data collected.

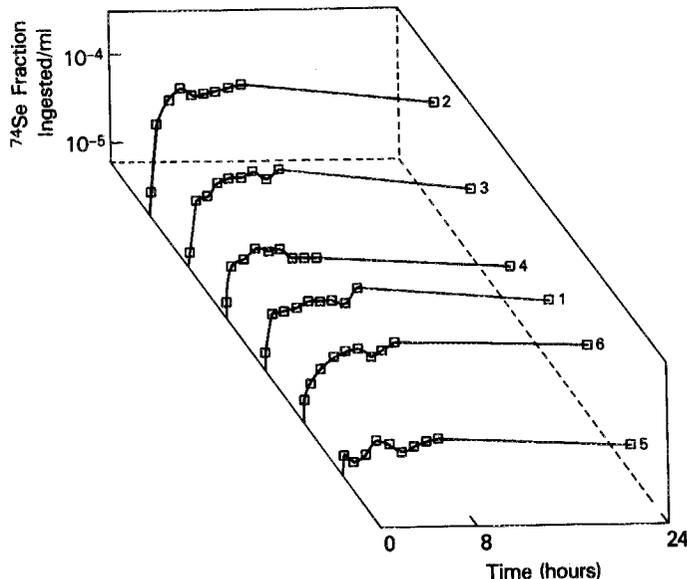


FIG. 4. Plasma ^{74}Se data for 1st 24 h after dosing. To illustrate patterns observed in data, data for each subject were normalized to 30-min sample and connected by lines. Numbers refer to subjects. For clarity of presentation, curves are arranged in order of increasing response (relative to 30-min sample), front to back.

From P3, label can be excreted in the urine or can move into a large, slowly turning-over pool labeled "tissues."

The very large and very slowly turning-over pool resulting from uptake by P3 may represent the peripheral tissues and is simulated by two compartments that exchange with each other. Label flows from P3 to T1 and from T1 to both P4, a fourth plasma pool, and a tissue pool T2, which serves as a pool in which label is sequestered. The fourth plasma pool is reflected in the long, gradually declining tail of the plasma curve, which likely represents material emerging very slowly, after a long delay, from the peripheral tissues. P4 probably contains the final metabolic products moving through the plasma before clearance into the urine.

Material from each of the plasma pools appears in the fractional urine curve, as shown in fractional urine data for *subject 2* (Figure 2B, top inset). This subject was chosen because his fractional urine data exemplify the patterns seen in all subjects. An initial spike in the urine curve comes from P1 and a second from P2. P3 contributes small amounts to the urine relative to those contributed by P1 and P2; its contribution can be seen, for this subject, between 48 and 96 h. The long tail of the urine curve corresponds to the long tail of the plasma curve and represents material cleared from P4.

The experimental data points for two subjects, as well as the curves predicted by the selenite model (indicated

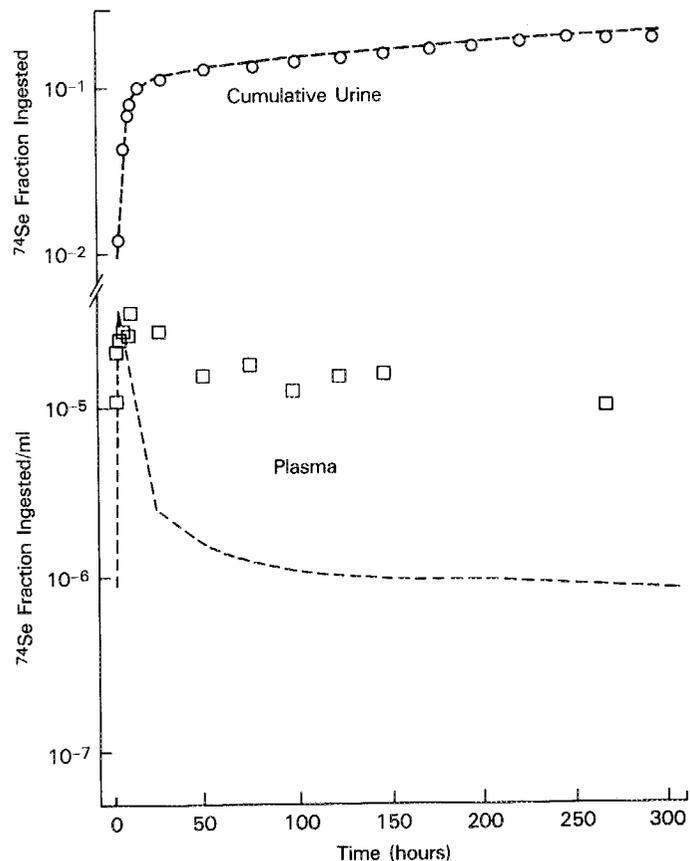


FIG. 5. Data for *subject 1* with theoretical plasma and cumulative urine curves generated using intermediate model. Dashed lines indicate simultaneous fits to plasma and cumulative urine data obtained when parameters were adjusted to give an adequate fit to cumulative urine data.

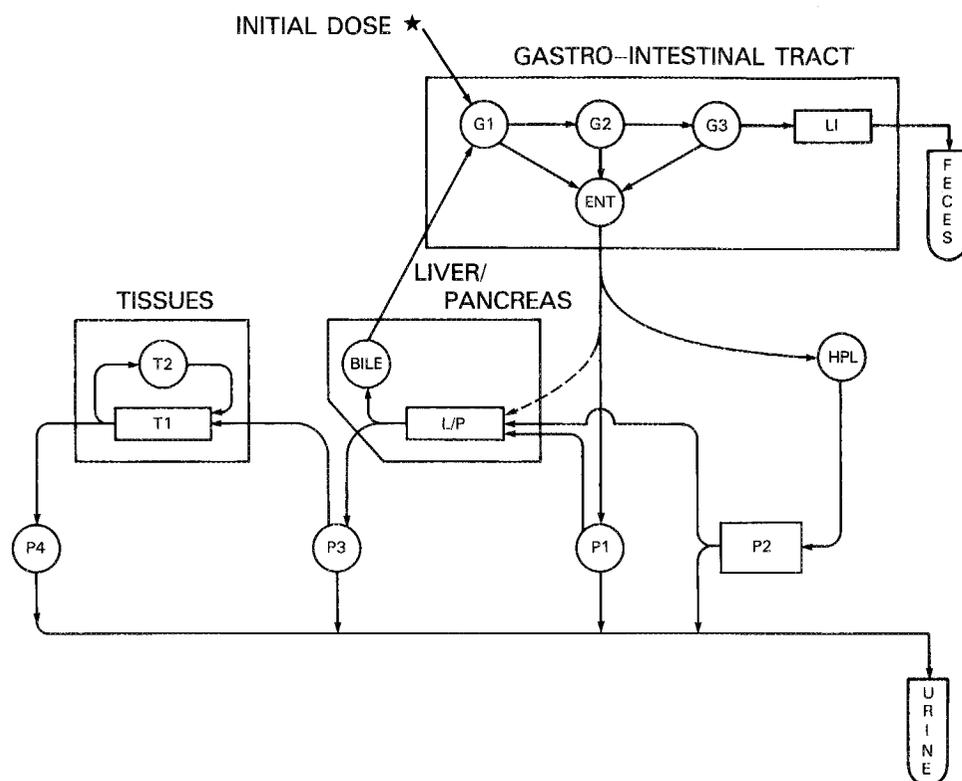


FIG. 6. Selenite model, a kinetic model for selenite metabolism. Arrow with an asterisk indicates site of entry of oral tracer Se. Arrows between compartments represent pathways of fractional transport. Compartments depicted as rectangles represent delays. G1, G2, G3, 3 gut compartments, probably small intestine; ENT, enterocytes (intestinal cells); HPL, pool in hepatopancreatic subsystem or lymphatic system (see text); L/P, liver and pancreas; LI, large intestine; T1, T2, peripheral tissues, in large part skeletal muscle. Feces and urine pools are drawn in shape of test tubes to represent fractional (single) collections. Model includes absorption distributed along gastrointestinal tract, enterohepatic recirculation, 4 kinetically distinct plasma pools P1-P4, a subsystem consisting of liver and pancreas, and a slowly turning-over tissue pool.

by dashed lines), are shown in Fig. 2. The plasma, urine, and fecal curves for these subjects, as well as for the remaining four subjects, show no systematic deviations from the experimental data.

Mean residence times are shown for several pools in Table 4, and corresponding fractional catabolic rates are given in Table 3. Residence times vary widely; for the four plasma components, they range from >0.5 h to nearly 12 days. Residence time in the liver and pancreas subsystem is much longer, ranging from 4 to 41 days. Residence time is far longer, between 115 and 285 days, for the tissue compartments.

DISCUSSION

In this section, we first discuss assumptions underlying the model. We then interpret the model and discuss its implications, addressing in particular absorption, recirculation, the four plasma pools, and the liver-pancreas and tissue pools. We then use the model to predict excretion and retention of the tracer dose after 90 days and to illustrate the effects of multiple hypothetical daily doses of tracer on the plasma and tissue levels for a single subject. Finally, we touch briefly on implications of the model for cancer prevention.

Assumptions and constraints. A major assumption is that subjects are in steady state. The linearity of tracer kinetics depends on the underlying system being in steady state (2); if the rates at which a substance moves through a system are changing, the observations reflect not only the rate at which the substance itself moves from one compartment to another but also the changes in the rate. To meet this requirement, the tracer dose should be small relative to the amount of the substance

in the diet, so as not to change the steady-state input to the system under study. In this study, subjects were fed a constant diet furnishing $87 \mu\text{g}$ of Se/day for 3 days before dosing. A dose of $200 \mu\text{g}$ of ^{74}Se , large relative to the dietary intake, was given in addition to the dietary Se. Without prior knowledge of how the metabolic system changes in response to an increase in Se intake, we began with the assumption that no change takes place and built the compartmental model based on this assumption. If there were any underlying changes in the system because of perturbations caused by the tracer, the fractional rate constants could be somewhat smaller or larger than if the system had been studied in a true steady state, but the structure of the model would probably not be affected.

There were two other possible, but less likely, sources of perturbation. As mentioned earlier, three subjects were given $200 \mu\text{g}$ of unlabeled Se daily on days 3-5 and 9-11, as part of a separate study. There were no apparent differences in the tracer data between these subjects and those who did not receive additional Se. The model predicts that by day 4 most of the retained tracer is in the tissues and is probably unaffected by additional inputs. A study in rats, however, showed possible effects on the turnover of tissue stores of Se due to changes in dietary Se intake (9). A second potential problem is that each subject received the same amount of dietary Se daily, so that intake per kilogram body weight varied between subjects. The effect of differential intake on the tracer data was probably inconsequential, because absorption and the immediate disposition of the label occurs in the first 8 h and, after that, the relative mass of the slowly turning-over body pools is very large compared with dietary intake levels.

TABLE 3. Parameters derived from selenite model

	Subject No.						Means \pm SE
	1	2	3	4	5	6	
<i>Absorption and fecal excretion</i>							
$L(G2,G1)$, h^{-1}	0.747	0.911	0.816	0.720	2.27	0.512	0.996 \pm 0.260
$L(G3,G2)$, h^{-1}	0.219	0.258	1.20	1.10	2.58	0.750	1.02 \pm 0.345
$L(ENT,G1)$, h^{-1}	0.213	0.009	0.384	0.380	0.826	0.238	0.342 \pm 0.112
$L(ENT,G2)$, h^{-1}	0.741	0.662	0.001	0.001	0.525	0.001	0.322 \pm 0.146
$L(ENT,G3)$, h^{-1}	0.020	0.304	0.900	0.878	2.45	0.564	0.853 \pm 0.348
$L(LI,G3)$, h^{-1}	0.795	0.096	0.100	0.055	0.135	0.110	0.215 \pm 0.116
Delay in LI, h	20.8	25.0	21.0	14.0	41.0	43.0	27.5 \pm 4.82
Delay in G1, G2, G3, h	1.042	1.087	0.833	0.909	0.323	1.333	0.060 \pm 0.016
<i>Urinary excretion</i>							
$L(urine,P1)$, h^{-1}	0.297	0.045	0.120	0.135	0.270	0.190	0.176 \pm 0.039
$L(urine,P2)$, h^{-1}	0.174	0.005	0.003	1.80	0.120	0.009	0.352 \pm 0.291
$L(urine,P3)$, day^{-1}	0.120	0.264	0.199	0.001	0.204	0.211	0.167 \pm 0.038
$L(urine,P4)$, day^{-1}	0.106	0.084	0.118	0.120	0.091	0.118	0.106 \pm 0.006
<i>Plasma components</i>							
Delay in ENT, h	0.036	0.139	0.038	0.050	0.036	0.060	20.8 \pm 3.28
Fraction to HPL*	0.174	0.333	0.162	0.178	0.185	0.382	0.236 \pm 0.039
$L(L/P,ENT)$, h^{-1}	0.00	0.00	0.00	0.85	0.00	0.00	NA
$L(L/P,P1)$, h^{-1}	1.75	0.90	1.90	1.30	3.90	1.70	1.910 \pm 0.425
$L(P2,HPL)$, h^{-1}	0.960	0.203	1.50	0.750	3.80	0.375	1.26 \pm 0.540
Delay in P2, h	4.35	8.00	5.10	3.30	3.00	2.65	4.40 \pm 0.810
<i>Liver-pancreas subsystem</i>							
$L(G1,bile)$, day^{-1}	0.119	0.034	0.024	0.017	0.036	0.031	0.044 \pm 0.015
$L(bile,L/P)$, h^{-1}	4.03	4.56	6.20	6.80	5.74	3.36	5.16 \pm 0.547
$L(P3,L/P)$, h^{-1}	3.97	3.44	3.80	3.20	2.26	4.64	3.55 \pm 0.328
Fraction to bile†	0.504	0.570	0.620	0.680	0.717	0.420	0.585 \pm 0.045
Delay in L/P, h	5.8	6.7	5.7	5.5	4.1	6.1	5.65 \pm 0.345
<i>Tissues</i>							
$L(T1,P3)$, h^{-1}	0.065	0.105	0.060	0.055	0.016	0.095	0.066 \pm 0.013
Fraction recirc‡	0.796	0.709	0.692	0.685	0.571	0.840	0.716 \pm 0.038
$L(P4,T1)$, h^{-1}	0.385	0.370	0.800	0.920	0.600	0.190	0.544 \pm 0.114
Delay in tissues, h	25.0	11.0	30.5	30.0	77.7	15.0	31.5 \pm 9.79

$L(I, J)$, fractional rate of flow from compartment I to compartment J ; ENT, enterocyte; LI, large intestine; HPL, hepatopancreatic or lymphatic compartment; L/P, liver and pancreas; P1-P4, 1st through 4th plasma components; G1-G3, gut compartments; T1, T2, peripheral tissue compartments; NA, not applicable. * $L(HPL,ENT)/[L(HPL,ENT) + L(P1,ENT) + L(liver,ENT)]$; † $L(bile,L/P)/[L(bile,L/P) + L(P3,L/P)]$; ‡ fraction recirculated: $L(T2,T1)/[L(P4,T1) + L(T2,T1)]$.

Absorption and enterohepatic recirculation. In humans, selenite is rapidly absorbed, as is apparent from the appearance of label in the plasma at 30 min. Absorption occurs rapidly in the model; Fig. 8 shows a simulation of the appearance and disappearance of tracer in the first gut compartment when multiple doses are given. The steepness and narrowness of the spikes reflect primarily the rapid removal of label through absorption. Levels preceding introduction of each new dose increase as a result of enterohepatic recirculation. Absorbed label is quickly excreted in the urine. We observed a spike in the urine data at 2-4 h, which corresponded to maximum excretion for some subjects. In a study in which a single oral dose of 10 μ Ci of 75 Se as selenite was given to three young women and specific activity was measured in the plasma, urine, and feces for a 14- to 19-wk period, Thomson and Stewart (33) observed that maximum excretion of radioactivity in the urine occurred within 2 h of dosing.

Absorption is modeled as being distributed along a portion of the gastrointestinal (GI) tract, probably the small intestine and possibly the stomach. Using ligated intestinal segments in rats, Whanger et al. (34) showed

that selenite is absorbed from all segments of the small intestine and that absorption from the duodenum is slightly greater than from other segments. While it has been shown that selenite is not absorbed from the stomach in the rat or in swine (34), it is not known whether this is the case in humans.

A pathway returning absorbed label to the gut was required to explain the appearance of label in the feces throughout the study period. Others, observing the continued appearance of labeled selenite in the feces over long study periods, have suggested that this indicates that absorbed label is excreted in the feces (16, 33). The selenite model hypothesizes a pathway from the liver and pancreas back to the GI tract, representing enterohepatic recirculation. This implies that tracer would be found in the pancreatic juices and/or the liver bile. Minor amounts of Se have been found in rat bile (22). Se has been used as a scanning agent for the pancreas, although as selenomethionine rather than as selenite. Based on the current model, it appears that humans excrete substantially more Se into the bile and the pancreatic juices than do rats. In our subjects, a significant fraction of the

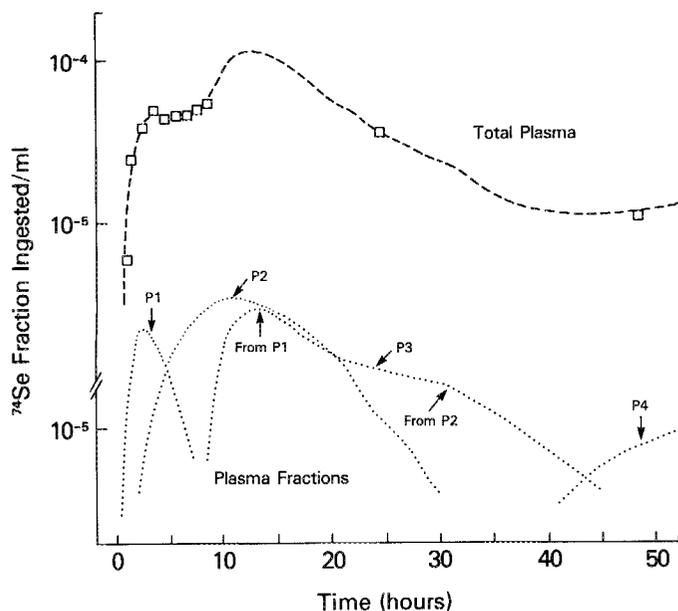


FIG. 7. Plasma curves calculated using selenite model for *subject 2*. Lower set of curves (dotted lines) shows 4 plasma components predicted using selenite model which, when added together, form predicted plasma curve (dashed lines) passing through observed data points. Third component, P3, is made up of 2 subcomponents, inputs to liver first from P1 and, after a delay, from P2.

TABLE 4. ^{74}Se mean residence times (in days)

	Subject No.						Means \pm SE
	1	2	3	4	5	6	
Plasma average	2.2	2.4	1.8	1.7	1.6	2.2	2.0 ± 0.1
P1	0.02	0.04	0.02	0.03	0.01	0.02	0.02 ± 0.004
P2	0.18	0.33	0.21	0.14	0.12	0.11	0.18 ± 0.03
P3	0.60	0.36	0.61	0.76	1.74	0.40	0.74 ± 0.21
P4	9.5	11.9	8.5	8.3	11.0	8.5	9.6 ± 0.6
L-P*	4	17	26	41	20	14	20 ± 5
Tissues†	274	255	121	115	285	278	221 ± 33

* Liver-pancreas subsystem; † peripheral tissues.

tracer that entered the liver and pancreatic subsystem was recirculated through the bile (Table 3). The fraction of material that recirculates back to the gut originating in the pancreas vs. that originating in the liver is not known in either rats or humans.

The presence of recirculated label in the feces complicates the process of estimating absorption. Absorption has been defined as the fraction of the element that is removed from the intestinal lumen and transported to the blood circulation, without return to the gastrointestinal tract during the time necessary to complete evacuation of the unabsorbed label in the fecal excretion (17). However, because some label may be resecreted into the gut before all unabsorbed label is excreted, some fecal samples may contain both unabsorbed and absorbed resecreted label.

Methods of estimating Se absorption in tracer studies have attempted to take excretion of absorbed label into account. A method proposed by Lutwak (23) consists of fitting a line to the terminal portion of the cumulative fecal curve (that part of the curve characterized by a gradual as opposed to a rapid rise), extrapolating the line

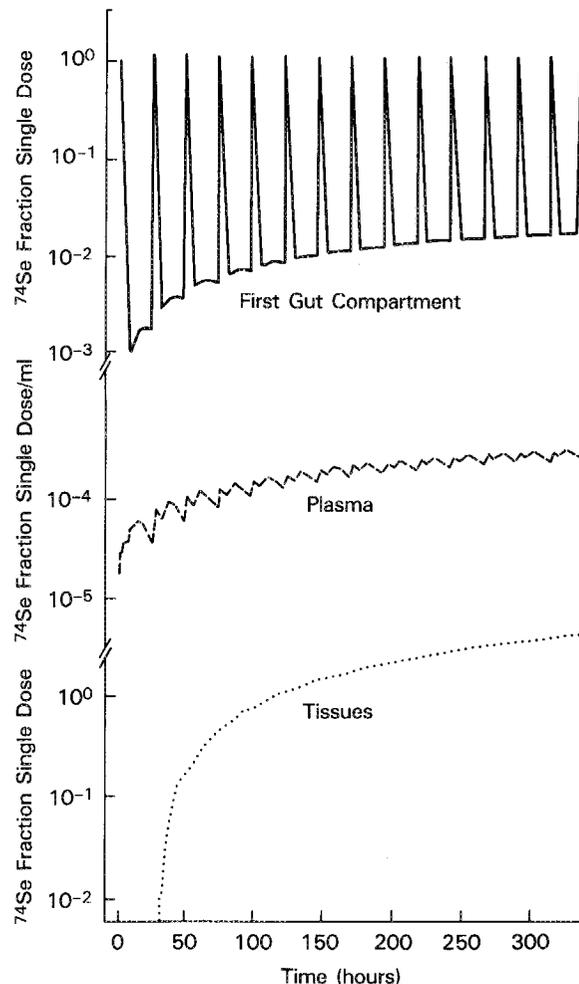


FIG. 8. Hypothetical dosing. Predicted concentration of ^{74}Se in plasma and predicted amounts of ^{74}Se in 1st gut compartment and tissue compartments for *subject 1* for the first 15 daily doses of label and assuming unchanging kinetics. Predictions were made using selenite model. Values for 30, 60, 180, and 250 days are given in Table 8.

to dose day and estimating absorption as the value of the line on that day. In the five-stool fecal pooling method, the total amount of label in five consecutive stool samples is taken as the unabsorbed portion (16). These methods depend on transit time and frequency of elimination. In a tracer study in which four young males were each given a single oral dose of $108.8 \mu\text{g}$ of ^{74}Se as $\text{H}_2^{74}\text{SeO}_3$, and label was measured in the feces for 32 days after dosing, Janghorbani et al. (16) found that these two methods gave very similar estimates of absorption. Using the selenite model, we estimated the fraction absorbed as that absorbed into the enterocyte; this estimate is unaffected by recirculated tracer. Estimates of absorption based on the methods described above are similar to those obtained from the model (Table 5). In our study, absorption was higher than that observed by Thomson and Stewart (33) (44–70% of dose) and by Janghorbani et al. (16) (48–63% of dose) but similar to absorption observed in a "two-period" study using labeled selenite: 76.0 ± 9.0 and $68.0 \pm 6.0\%$ (SE) of dose (15).

Plasma pools. Selenite is a highly reactive molecule that likely undergoes a complex series of reactions when

TABLE 5. Estimated absorption (%dose) of ⁷⁴Se

Method	Subject No.						Means ± SE
	1	2	3	4	5	6	
Fecal pooling	80.1	80.8	82.8	87.7	87.0	81.8	83.4±1.3
Lutwak's method	81.6	82.3	84.2	89.4	89.1	83.0	84.9±1.4
Selenite model	82.6	81.4	83.0	86.8	87.3	83.1	84.0±1.0

introduced into the body, probably resulting in the production of various species of Se, such as selenides, selenotrisulfides, and elemental Se. While it is recognized that the biological activity of Se is an expression of this element in a wide variety of compounds, and not the element per se, little is known about the pharmacokinetics of these individual compounds (10). Because of their low concentrations and chemical instability, only a few organoselenium compounds have been isolated from biological materials and reliably characterized (11). Using the techniques of compartmental modeling, we have separated plasma Se into four kinetically distinct fractions, probably different compounds, corresponding to the four plasma pools. Figure 7 shows these fractions, as predicted by the model, for a single subject, and Table 6 gives the times at which three of the four fractions peaked.

The first component, P1, may be material present in the blood traveling through the portal vein but not removed by the liver. As mentioned earlier, the first-pass effect is negligible, if it exists, in fasting subjects. It is possible that, under this study design, a small first-pass effect could not be detected. Alternatively, P1 may represent material that comes from a rapidly turning-over pool in the liver.

A second component, P2, appeared in the plasma 2–3 h after dosing and remained there for a period of at least 3–6 h. This second plasma pool may represent material taken up by the liver in the first-pass effect. If this hepatic pathway is present, it has a different delay time than the other hepatic pathway represented in this model. Alternatively, this pool may represent Se that is absorbed from the enterocyte into the lymphatic system, flowing up the thoracic duct and emerging, after a delay, into the great veins of the neck. Both the timing of the appearance of this component and the shape of the plasma curve are consistent with models for chylomicron and very low-density lipoproteins triglyceride kinetics (27). The existence of a pathway through the lymphatics is supported by the fact that Se has been found in lipoproteins (4).

TABLE 6. Predicted times of peak plasma levels of kinetically derived Se fractions

Plasma Fraction	Subject No.						Means ± SE
	1	2	3	4	5	6	
Σ*	10.5	10.9	9.5	11.2	10.4	14.1	11.1±0.6
P1	1.3	2.3	1.3	1.6	0.6	1.5	1.4±0.2
P2	6.1	10.0	5.1	4.1	4.5	5.1	5.8±0.9
P3	13.1	12.7	12.8	13.7	10.4	14.7	12.9±0.6

Peak value of 4th plasma component was not estimable at this sampling frequency. Times expressed in hours. * Sum of all 4 components taken together.

The model predicts that a third component, P3, remains in the liver for ~4–7 h before emerging. This theoretical component peaks at ~13.0 h (Table 6). The peaks representing maximum concentration occur between 9 and 14 h; this is in general agreement with Thomson and Stewart (33), who observed peak values at 7, 10, and 12 h, and with Janghorbani et al. (15), who found that peak serum enrichment occurred between 8 and 12 h after dosing with labeled selenite.

The plasma component represented by P3 is probably a protein or selenoenzyme secreted or excreted by the liver. Thomson and Stewart (33) refer to a "posthepatic" form of ⁷⁶Se and speculate that the rapid decrease in activity in the plasma after dose day may represent utilization by the tissues of material returned from the liver into the blood in a protein-bound form, or turnover of material incorporated into other rapidly metabolized serum proteins. We speculate that the label is secreted or excreted from the liver in the form of protein, possibly glutathione peroxidase, or other selenoproteins. Both inorganic and organic forms of Se are metabolized by animals into biologically active compounds, including selenocysteine, which has been identified as the form of Se in glutathione peroxidase (10). Other selenoproteins have been identified in rat plasma (35) and in rat liver and other tissues [for review see Combs and Combs (7)]. A study by Motsenbocker and Tappel (24) indicates that protein P, a Se-containing protein, is synthesized in rat liver and that it transfers Se from the liver to extrahepatic tissues.

The fourth plasma pool, P4, is fed by the large and slowly turning-over tissue pools. Thomson and Stewart (33) suggest that the final phase of the plasma curve represents long-term metabolism and reutilization of material with a slow turnover rate that is incorporated into serum proteins.

The multiplicity of Se plasma pools has been recognized in the literature. Thomson and Stewart (33) resolved plasma curves into three exponential components. We converted their reported half-lives to mean residence times.¹ For the first two components, these were 1.0–2.0 days and 7.5–13.1 days, similar to those for our third and fourth plasma components, which ranged from 0.4 to 1.7 days and from 8.3 to 11.9 days, respectively. Their third component, with a mean residence time of 100–111 days, probably reflects label coming out of the tissues and is shorter than that for our tissue compartment, 115–285 days.

The multiple peaks and valleys in the observed urine data represent Se components in the urine arising from the four plasma components. In animals, intermediary metabolism of Se characteristically involves reduction and methylation resulting in the excretion of urinary or respiratory metabolites that include the trimethylselenonium ion and dimethylselenide (10). Little is currently known about the Se compounds found in human urine. Trimethylselenonium ion is the only species that has been studied extensively; it accounts for only a small fraction of total urinary Se (10).

¹ Half-life ($t_{1/2}$) is related to residence time (RT) as follows: $t_{1/2} = -RT(\ln 0.5)$, or, approximately, $t_{1/2} = 0.693 RT$.

Liver and tissue pools. According to animal studies, the liver is an important Se reservoir. Behne and Wolters (1) reported that the liver accounts for ~32% of the total body Se in the rat. No comparisons can be made with values from the model, because we cannot distinguish individual organs in the subsystem designated liver-pancreas, which may include other organs fed by the splanchnic bed. Mean residence times for this subsystem ranged from 4 to 41 days for the six subjects (Table 4).

The peripheral tissues represent a large and very slowly turning-over pool which probably includes muscles, bones, kidneys, and other organs. About 46% of the total body Se content is in the muscle (0.24 mg/kg) of North Americans (19). It was not possible to differentiate specific tissues or their residence times in this study. Mean residence times for this large pool represent a weighted average of the residence times of the various tissues; these range from 115 to 285 days. This long period has profound implications for supplementation. As described below, if the kinetics were to remain constant under supplementation, very large stores of Se could build up in the tissues.

Parameter variations. Means and standard errors are shown for all parameters, but it should be noted that six subjects are an insufficient number to generate reliable population estimates. Overall, the similarities between the values of the rate constants are impressive. Variations in the parameter values represent variation from subject to subject, changes in the environment while the study was underway, and measurement errors. For example, large differences in individual values of fractional rates of flow $L(ENT,G2)$ and $L(ENT,G3)$ probably represent short-term changes in bowel motility. On the other hand, there is no readily available physiological rationale to explain the large variations in $L(urine,P2)$, and $L(urine,P3)$, and it is unlikely that these reflect incomplete collections. Variations such as these can best be assessed by repeating the study on the same subjects; however, such measurements are not currently available.

Predictions. Ninety days was the proposed washout period for a split-unit study under consideration in which two doses of selenite were planned. Using the selenite model and parameters shown in Table 3, we simulated retention and excretion for 90 days in response to a single dose of selenite. After 90 days, ~35% of the dose was retained in the body and ~65% was excreted, 38% in the urine, and 27% in the feces (Table 7).

Several estimates have been made of the half-lives associated with long-term retention of Se given as selen-

ite. Cavalieri et al. (5) estimated retention of an intravenous dose of ^{75}Se in three patients, followed for a period of 91–204 days, by a single exponential decay curve with a half-life of 65 days for the 75% of the dose not excreted in the first 3 days. Jereb et al. (18) followed 26 patients given intravenous injections of radioselenite for up to 517 days; they estimated that ~40% of the dose had a half-life of ~20 days, whereas 48% had a half-life of ~115 days. Thomson and Stewart (33) resolved whole body counting measurements into three exponential phases, with half-lives ranging from 0.8 to 1.4 days for the first phase, 4–14 days for the second, and 92–143 days for the third. Based on total excretion of ^{74}Se over a 32-day period, Janghorbani et al. (16) estimated retention by the sum of two exponentials with half-lives of 2.4 ± 0.3 and 162 ± 9 days. We calculated half-lives from the mean residence times shown in Table 4. Half-lives ranged from 1.1 to 1.7 for the weighted average of the four plasma pools, similar to those calculated by Thomson and Stewart (33) for the first exponential component. Half-lives for the peripheral tissues probably correspond to the last, and longest, exponential components reported by others. These ranged from 80 to 198 days and encompass the values reported above.

Using the selenite model and parameters given in Table 3, we simulated daily supplementation of 200 μg of labeled Se as sodium selenite for a period of 250 days for *subject 1*, and estimated increases in plasma concentration and in levels in the peripheral tissues. This simulation might represent the situation in which supplementation is undertaken for chemopreventive purposes. However, it does not take into account dietary Se, either in relation to daily intake or to accumulations already present in the plasma or the tissues. More importantly, the predictions are an extrapolation of the kinetics of a single dose and do not reflect possible homeostatic regulation, which would likely reduce absorption and increase excretion, thus resulting in less accumulation. As such, these estimates represent an upper bound and they exceed values reported in the literature.

Predicted increases in tracer levels in the plasma and the tissues are given in Table 8 and are shown graphically

TABLE 8. Predicted increases in plasma concentration and in amount in tissues after daily supplementation of 200 μg of ^{74}Se for subject 1. Numbers reflect increases over levels due to dietary intake

Supplementation, days	Plasma		Tissues	
	ng/ml	Fraction of daily dose/ml*	mg	Multiple of single dose†
30	62.5	3.1×10^{-4}	1.8	9.2
60	82.0	4.1×10^{-4}	3.9	19.6
180	133.0	6.7×10^{-4}	10.7	53.5
250	156.0	7.8×10^{-4}	13.7	68.5

* Numbers are in units of fraction of daily dose (200 μg) per ml and are given for purposes of comparison with units of ordinate of middle (plasma) curve in Fig. 8, e.g., 62.5 ng/ml = 3.1×10^{-4} fraction of dose/ml \times 200 $\mu\text{g}/\text{dose} \times 1,000 \text{ ng}/\mu\text{g}$. † Values are equivalent to multiples of a single dose (e.g., 1.8 mg = 9.2 doses \times 200 $\mu\text{g}/\text{dose}$) and are given for purposes of comparison with units of the ordinate of bottom curve (tissues) in Fig. 8.

TABLE 7. Estimated cumulative excretion and retention of dose (%dose) after 90 days

	Subject No.						Means \pm SE
	1	2	3	4	5	6	
Cumulative excretion							
Urine	40	29	35	43	50	32	38.2 \pm 3.1
Feces	27	29	28	26	25	24	26.5 \pm 0.8
Total	67	58	63	69	75	56	65 \pm 2.9
Retention	33	42	37	31	26	44	35 \pm 2.8

A washout period of 90 days was under consideration in a split unit study in which 2 doses of Se would be administered.

for the first 15 days in Fig. 8. As expected from the residence times of the plasma and tissue compartments (Table 4), levels are still increasing at the end of 15 days. Daily oscillations in the plasma curve are visible for the first several days of hypothetical dosing; these dampen on this logarithmic scale as levels increase. Tissues levels begin rising at ~2 days, and calculated levels are still rising at 250 days. While the rate of increase slows over the whole period, at 250 days the additional total body accumulation for this subject is still <50% of the calculated asymptotic value of 30 mg, which would be achieved at about five half-lives, or ~1.4 yr.

There is a very wide range in reported increases in plasma levels resulting from supplementation. In an 11-wk supplementation study in Finland in which 10 men were given a daily dose of 200 μg of selenium as selenate, another inorganic form of selenium, plasma levels increased by ~61% (21). Several supplementation studies have been performed in New Zealand. In an experiment in which 20 patients suffering from muscular complaints were given 500 μg of sodium selenite per day for 20 days, blood Se levels almost doubled during the first week. Se levels remained elevated until gradually decreasing during the last week of the study (31). In a second study, a single subject received a daily supplement of 100 μg of selenite for 10 wk; blood Se plateaued at levels about one-third above base line and then slowly decreased (28). In a third study, in which four subjects were given 500 μg of selenite daily for 4 wk, a doubling of plasma Se levels was observed (32). In comparison, in our simulation, after 60 days, supplementation would add ~82 ng/ml to plasma levels (Table 8). Based on an observed level of 133 ng/ml of (dietary) Se already present in the plasma of this subject, this implies an increase of ~62%. As noted above, the doses simulated are in addition to the usual dietary intake, and predictions are based on the assumption that the kinetics remain unchanged. The dynamic mechanisms of possible homeostatic regulation (i.e., changes in absorption, distribution, and excretion) of selenite may result in lower tissue retention. The details of the relationship between rate constants and tissue levels need to be characterized in future experiments if human dosing with Se is envisaged.

Conclusions. Using kinetic modeling techniques, we have separated the metabolism of Se into multiple components. If, as we have suggested, the kinetically distinct plasma components represent chemically different forms of Se, each may have a specific, probably different, mechanism of action in relation to nutritional requirements, toxicity, and cancer prevention. If Se is an effective cancer-prevention agent, its efficacy may not be uniform throughout the body; the amount and form reaching various organs or tissues may be of considerable research interest. Studies can be planned to identify those particular forms that show the greatest potential beneficial effect and to further characterize their particular kinetics.

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