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Metabolism, Cellular Actions, and Cytotoxicity of Selenomethionine in Cultured Cells†

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ABSTRACT

Selenomethionine metabolism and the biochemical basis for its cytotoxicity were analyzed in cultured human and murine lymphoid cells. The metabolic pathways were also addressed, using purified mammalian enzymes and crude tissue extracts. Selenomethionine was found to be effectively metabolized to *S*-adenosylmethionine analog, and that analog was further metabolized in transmethylation reactions and in polyamine synthesis, similarly to the corresponding sulphur metabolites of methionine. Selenomethionine did not block these pathways, nor was there a specific block on the synthesis of DNA, RNA, or proteins when added to the culture medium. Selenomethionine showed cytotoxicity at above 40 μ*M* levels. Yet, low selenomethionine levels (10 μ*M*) could replace methionine and support cell growth in the absence of methionine. Selenomethionine

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toxicity took place concomitantly with changes in *S*-adenosylmethionine pools. D-form was less cytotoxic than L-form. Methionine concentration modified the cytotoxicity. Together, this indicates that selenomethionine uptake and enzymic metabolism are involved in the cytotoxicity in a yet unknown way.

INTRODUCTION

Biological methylation reactions are important regulators of metabolism. Methylation of cytosine in DNA regulates transcriptional activity of genes. Thus, biological methylation reactions are of utmost importance in mammalian cell differentiation, and possibly also in malignant transformation. The methyl donor in these reactions is *S*-adenosylmethionine (AdoMet). Selenomethionine (SeMet), a close analog of methionine (Met), is capable of forming a selenium analog of AdoMet (*1,2,3*). Tracer studies have indicated that SeMet is metabolized, possibly via the methionine metabolic pathways, resulting finally in the formation of dimethylselenide and trimethylselenonium (*4*). However, these final products differ from those of methionine catabolism. Details of the metabolic processes involved are still poorly known. Impaired synthesis of AdoMet (*5*) or inhibition of its utilization in DNA methylation (*6*) have been indicated in selenite toxicity. The role of adenosylselenomethionine (AdoSeMet) in methylation reactions has not been studied in detail. Also, little is known about the use of AdoSeMet in the synthesis of polyamines that are closely involved with the regulation of cell growth.

In this report, SeMet metabolism via the pathways of transmethylation and polyamine synthesis, and its effects on cellular macromolecule synthesis, have been elucidated in cultured cells.

MATERIALS AND METHODS

Cell Culture

Culture of K-562 (human chronic myelocytic leukemia) and R1.1 (murine T-lymphoma) cells has been previously described in detail (*7*). Briefly, the cells were maintained in suspension culture in RPMI 1640, supplemented with 10% heat-inactivated fetal bovine serum (Gibco, lot 10G7572F), penicillin (100 U/mL), and streptomycin (100 μg/mL). The methionine concentration in the medium was varied, using an RPMI 1640 Select-Amine kit. All cell culture reagents were from Gibco (Chagrin Falls, OH). All cell lines were from American Type Culture Collection (Rockville, MD). L-SeMet and DL-SeMet were from Sigma Chemical Co. (St. Louis, MO). Unless stated otherwise, L-SeMet was used in all cell culture experiments.

Selenium Content of Cell Culture Media

Selenium content was measured by electrothermal atomic absorption spectrometry (*8*). The fetal bovine sera and the RPMI 1640 medium contained 58 ± 15 n*M* and 23 ± 3 n*M* selenium, respectively. Since fetal bovine sera was added to the growth medium to 10% concentration, the final selenium levels in the culture media were about 30 n*M*.

Toxicity Assays

Growth inhibitory action of selenium-containing compounds was assayed in microplate cultures. Serial two-fold dilutions of growth inhibitory compounds were added to cell cultures at an initial density of 1×10^5/mL. After 72 h in culture, living cells (excluded erythrocin B) were counted manually. The reported values are means of at least two independent determinations.

Macromolecule Labeling

The rate of macromolecule synthesis was determined by isotope-labeling of cultures treated with 0–2.5 m*M* SeMet for 24 h or 48 h. The assays were done in triplicate, with serial dilutions of SeMet on 96-well plates. L-[4,5-^{3}H]leucine (1 μCi/well), [6-^{3}H]thymidine (1 μCi/well), or [2-^{14}C]uridine (0.5 μCi/well) dissolved in a small volume of culture medium (20–50 μL) were added to the wells containing 200 μL of culture medium. After 3 h, labeling was stopped by harvesting cells with Multimash 2000 (Dynatech Lab., Chantilly, VA). The cells were collected on glass fiber filters by washing the plate with phosphate buffered saline (PBS; 150 m*M* NaC1, 10 m*M* phosphate buffer, pH 7.4). Thereafter, cells labeled with leucine were washed with 10% trichloroacetic acid (*9*). Uridine labeled cells were washed with PBS, and thymidine labeled ones with H_2O. The glass fiber filters were counted in ACS scintillation fluid (Amersham, UK).

Cell Harvesting

For measuring methionine adenosyltransferase activity, washed cells (4×10^7) were frozen and thawed three times in distilled water (400 μL), and centrifuged at 14,000*g* for 5 min in Eppendorf tubes. The supernatant was used after dialysis for the activity assay.

For metabolite analysis, the cultured cells were collected by centrifugation at 700*g* for 5 min at 4°C. After removal of supernatants, the cells were washed once in ice-cold PBS. The pellets were extracted with ice-cold 0.4 *M* perchloric acid for 15 min. After centrifugation, the supernatant fractions were neutralized with Alamine-Freon (*7*), and subjected to HPLC.

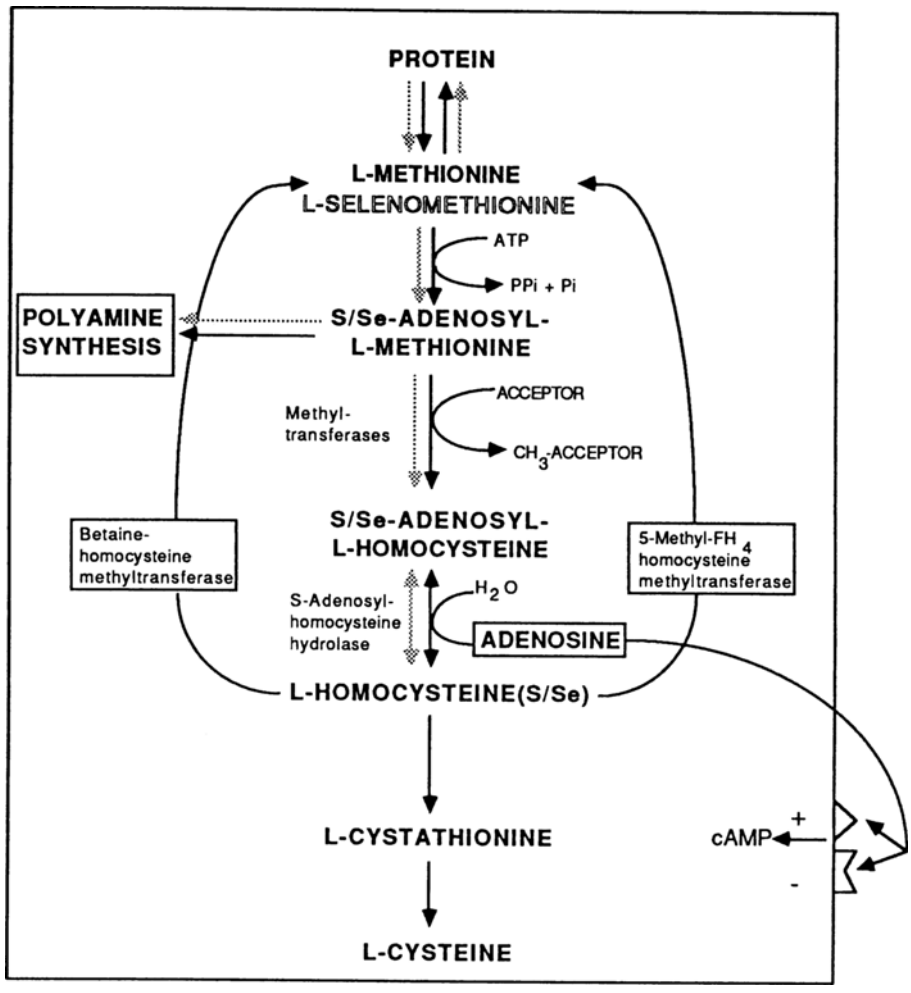

Fig. 1. Known methionine metabolic pathways in mammalian cells are shown with black arrows. In this study, possible metabolic pathways for L-Se-Met marked with shaded arrows were studied. Formation of cAMP takes place on the cell membrane under influence of e.q. adenosine receptors.

Enzyme Assays

Methionine adenosyltransferase activity was assayed as described previously by Kajander et al. (*7*). Assays for histamine-*N*-methyltransferase (*10*). *S*-adenosylhomocysteine hydrolase (*11*), *S*-adenosylmethionine decarboxylase (*12*), spermidine synthase (*13*), and spermine synthase (*14*) were also performed according to the published procedures.

Table 1
AdoMet and AdoSeMet Pools in R1.1. Cells

Addition (μ*M*)		AdoMet	AdoSeMet
Met	SeMet	pmol/10^6 cells	
—	—	24.3	—
10	—	31.2	—
—	10	33.3	2.0
40	—	40.2	—
—	40	32.3	11.1
100	—	45.9	—
—	100	15.8	17.0

Cells were incubated for 16 h with the indicated Met or SeMet addition into the normal culture medium (containing 100 μ*M* Met). Cells were harvested and AdoMet pools were assayed as described in the Methods. Values are means of two determinations.

HPLC

Sulphur- and selenium-containing metabolites of SeMet were determined with a modification (*15*) of the HPLC method of Wagner et al. (*16*). ATP levels were determined using the Nucleosil HPLC-column (Vydac, Hesperia, CA), according to the instructions of the manufacturer.

Preparation of SeMet Metabolites

AdoSeMet, adenosylselenohomocysteine (AdoSeHcy), decarboxylated AdoSeMet, and methylselenoadenosine (MeSeAdo) were enzymatically prepared and purified, as previously described by Kajander et al. (*15*).

RESULTS

Metabolic Routes of Selenomethionine in Cells

We have previously synthesized SeMet metabolites by using enzymes involved in the pathways of transmethylation and polyamine synthesis (*15*). As described below in detail, we found that the mammalian enzymes metabolizing the sulphur metabolites of methionine (Fig. 1) could utilize the corresponding selenium analogs as their substrates (Fig. 1, shaded arrows).

Utilization in Protein Synthesis

SeMet is known to be incorporated into proteins as a replacement for methionine (*17,18*). This was evidently true in R1.1 and K-562 cells as well, because these cells could be grown for at least one month in a medium where methionine was replaced by subtoxic (10 μ*M*) levels of SeMet. Growth rate was about half of the normal rate under such culture

Table 2
Methionine Adenosyltransferase Activity
in K-562 Cell Extracts

SeMet (μM)	Methionine adenosyltransferase pmol/(min × mg)
—	169 ± 0.7
10	198 ± 6.6
40	152 ± 8.1
100	182 ± 12.6

The extracts were prepared from cells incubated for 16 h in the presence of the indicated SeMet concentrations in the culture medium (contains 100 μM Met). The cells were thoroughly washed, and frozen and thawed three times before centrifugation in Eppendorf tubes (14,000*g* 5 min). Supernatant fractions were used for enzyme activity analysis. Values given are means ± SD of four experiments.

conditions. Cell size and protein concentrations were similar to cells cultured with methionine, indicating that protein synthesis and degradation of proteins containing SeMet were not imbalanced.

Effect of SeMet on AdoMet and AdoSeMet Pools, and on Methionine Adenosyltransferase Activity

As shown in Table 1, AdoMet pools increased in R1.1 cells cultured in a medium with increasing concentrations of medium methionine. Supplementation of the medium with SeMet resulted in a formation of increasing pools of AdoSeMet concomitantly with decreased AdoMet pools. Methionine adenosyltransferase activity was not affected in cells treated with SeMet (Table 2). Thus, SeMet and methionine competed for the same enzymic reaction in the living cell, as well as in cell extracts (Table 3). The apparent "inhibition" of methionine adenosyltransferase in R1.1 extracts was identical with both L-SeMet and L-methionine because of dilution of the radiolabeled methionine. Both DL-SeMet and DL-methionine were less "inhibitory" because the D-form is not an effective substrate (data not shown). DL-Selenoethionine proved to be a poor substrate, too. AdoMet caused stronger product inhibition of the transferase activity than did AdoSeMet.

Transmethylation and Degradation of AdoSeHcy

AdoSeMet was found to be an excellent substrate for the purified rat kidney histamine-*N*-methyltransferase. K_m values for AdoMet and AdoSeMet were 1.1 and 0.9 μM, and V_{max} values 14.8 and 13.4 pmol/min, respectively, for the same enzyme preparation. We also tested whether AdoSeHcy is formed in crude rat liver preparations. Thoroughly dialysed (removes small molecules that are substrates for transmethylases) supernatant fraction of rat liver was first treated with 17 μM

Table 3
Inhibition of Methionine Adenosyltransferase
by Selenium-Containing Compounds

Compound	Concentration in the incubation mixture		
	750 μ*M*	150 μ*M*	30 μ*M*
DL-Selenoethionine	78 ± 2	89 ± 4	94 ± 3
DL-Selenomethionine	21 ± 2	73 ± 2	90 ± 5
DL-methionine	25 ± 3	56 ± 5	71 ± 3
L-Selenomethionine	15 ± 3	60 ± 2	86 ± 7
L-methionine	15 ± 1	47 ± 2	67 ± 2
AdoSeMet	85 ± 11	92 ± 3	97 ± 2
AdoMet	10 ± 1	48 ± 1	75 ± 4

The methionine adenosyltransferase activity was assayed in 76 m*M* Tris/HCl (pH 7.4) containing 40 m*M* KCl, 25 m*M* $MgCl_2$, 10 m*M* ATP, 2.5 m*M* β-mercaptoethanol, and 150 μ*M* L-methionine (4.9 μCi/μmol). Indicated compounds were added to the assay mixture 5 min before enzyme addition. Incubation was carried out at 37°C for 45 min and terminated by adding 25 μL of the reaction mixture to a P-81 phosphocellulose paper (diameter 2 cm). Phosphocellulose papers were then washed three times with 600 mL distilled water, dried, and counted for radioactivity. The values given are means ± SD (n = 3) and indicate percent of control activity determined in the absence of inhibitors.

erythro-9-(2-hydroxy-3-nonyl)adenine and 217 μ*M* arabinosyl adenine in order to inactivate AdoHcy hydrolase (*11*). Then, 0.24 m*M* AdoSeMet was added. Only a small fraction (less than 10%) of the added AdoSeMet degraded in 30 min to AdoSeHcy, a byproduct of transmethylation from AdoSeMet. When 2 m*M* glycine and 10 m*M* nicotinamide (substrates for glycine-*N*-methyltransferase and nicotinamide-*N*-methyltransferase, respectively) were added to the reaction mixture, over 90% of AdoSeMet was converted to AdoSeHcy during the 30 min incubation period. AdoSeHcy was hydrolyzed to adenosine and selenohomocysteine by purified (*11*) AdoHcy hydrolase. In cultured cells, no accumulation of either AdoSeHcy or AdoHcy was observed, indicating that both compounds are efficiently metabolized further.

Synthesis of Decarboxylated AdoSeMet, Polyamines, and MeSeAdo

Decarboxylated AdoSeMet was prepared from AdoSeMet using *E. coli* AdoMet decarboxylase, as described in reference 15. The reaction yielded about 20% of the decarboxylated derivate, a value similar to that obtained with AdoMet as the substrate. The mammalian enzyme proved also active in making decarboxylated AdoSeMet. In an experiment using the dialysed rat liver extract in incubation with 0.24 m*M* AdoSeMet for 30 min without substrates for transmethylation, decarboxylated AdoSeMet was formed, and was responsible for at least 70% of the AdoSeMet consumption (the rest being converted to AdoHcy and MeSeAdo).

Decarboxylated AdoSeMet was found to be an active propylamine group donor in the polyamine synthesis in vitro. Decarboxylated AdoSeMet was utilized by purified bovine spermidine synthase and

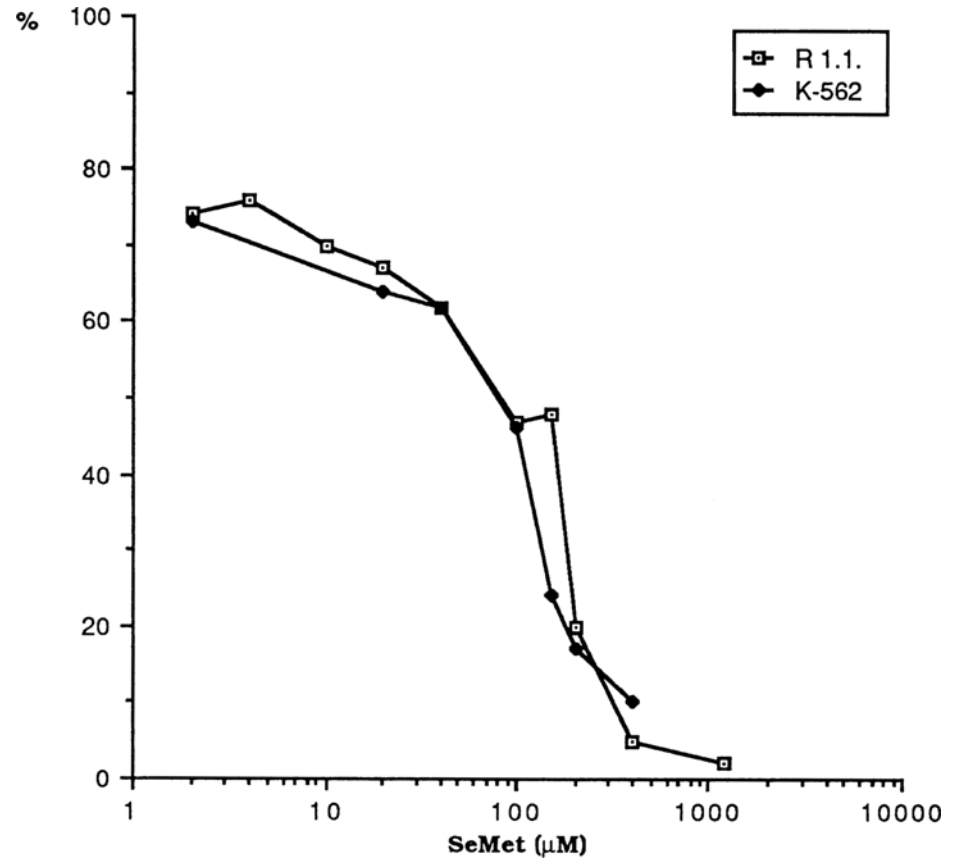

Fig. 2. Effects of SeMet on the growth of R 1.1 and K-562 cells. The cells were incubated for 72 h with the indicated additions of SeMet. Abscissa shows SeMet concentration and ordinate percentage of control growth without added SeMet.

spermine synthase comparably to decarboxylated AdoMet. The by-product MeSeAdo did not accumulate in cultured cells, again analogously with the sulphur compound, methylthioadenosine. Both of these compounds were also proved equally potent inhibitors of spermine synthase in vitro (data not shown).

Cytotoxicity of SeMet

Effect of SeMet on Cell Growth

SeMet showed a marked cytotoxicity at concentrations above 40 μ*M* in all cell lines studied. Fifty percent growth inhibition in a 3 d culture experiment was caused by 40–160 μ*M* addition of SeMet to a culture medium containing 100 μ*M* L-methionine (E. O. Kajander, manuscript in

Table 4
The Effect of L-SeMet on Macromolecule Synthesis by K-562 Cells

SeMet m*M*	Incorporation of radiolabel 24 h	48 h
	Thymidine	
0.000	1127 ± 116	1302 ± 207
0.039	1055 ± 47	1248 ± 156
0.078	951 ± 67	944 ± 83
0.156	830 ± 68	705 ± 75
0.31	673 ± 33	440 ± 83
0.625	634 ± 40	388 ± 44
1.25	630 ± 33	334 ± 16
2.5	763 ± 27	312 ± 35
	Leucine	
0.000	941 ± 200	1870 ± 311
0.039	977 ± 242	1200 ± 190
0.078	736 ± 106	898 ± 199
0.156	688 ± 165	662 ± 145
0.31	570 ± 53	490 ± 71
0.625	573 ± 90	401 ± 44
1.25	575 ± 106	392 ± 77
2.5	556 ± 118	212 ± 88
	Uridine	
0.000	5432 ± 1930	10818 ± 1541
0.039	5342 ± 1364	6471 ± 1203
0.078	4426 ± 761	4547 ± 975
0.156	3472 ± 538	3381 ± 535
0.31	2840 ± 514	2446 ± 183
0.625	2550 ± 609	1786 ± 133
1.25	2304 ± 434	1369 ± 199
2.5	2372 ± 375	1165 ± 186

preparation). Figure 2 shows the cytotoxicity curves for R1.1 and K-562 cells. The growth inhibition was identical for these two cell lines. Fifty percent growth inhibition was brought about by 90 μ*M* SeMet. SeMet toxicity increased when medium methionine concentration was lowered. Fifty percent growth inhibition was seen already at 40 μ*M* SeMet when cells were cultured in a medium containing only 10 μ*M* methionine. DL-SeMet was about half as toxic as *L*-SeMet.

Addition of SeMet at concentrations below 40 μ*M* to the culture medium of various hepatoma cell lines increased their growth rate and plating efficiency by even 50% (*19*). SeMet addition did not, however, increase the growth of lymphoid and erythroid cell lines.

Effects of SeMet on the Rate of Macromolecule Synthesis and ATP Levels

The rate of macromolecule synthesis by SeMet-treated K-562 cells was analyzed by the pulse-labeling technique (Table 4). At the highest SeMet dose of 2.5 m*M*, the incorporation of thymidine decreased by about 40%, of leucine by 40%, and of uridine by 55% within 24 h. By 48 h, the values were 75%, 90%, and 90%, respectively. Thus, no specific block in DNA, protein, or RNA synthesis took place at toxic SeMet levels. Met and SeMet additions did not affect cellular ATP levels of R1.1 cells, when tested after 16–24 h incubations.

DISCUSSION

The biochemical basis of SeMet toxicity is not known. Clarification of SeMet metabolic pathways and knowledge of their interrelationship to methionine pathways have been studied here. Such knowledge is of importance for understanding possible mechanisms for the toxicity, especially because methionine is involved as a precursor in transmethylation reactions, and in polyamine synthesis. These are of vital importance in the regulation of cell functions and growth.

Two different cell lines were selected as models for metabolic and toxicity studies of SeMet. As shown above, SeMet caused identical growth inhibition in both cell lines. SeMet was converted to AdoSeMet in cells as efficiently as methionine to AdoMet, indicating that methionine adenosyltransferase could utilize it as a substrate. Furthermore, SeMet addition to the culture medium did not influence the transferase activity and could support growth, and thus, be used in protein synthesis as a replacement for methionine. AdoSeMet was found to function well in three transmethylation reactions, and the byproduct AdoSeHcy was hydrolyzed to SeHcy. AdoSeMet was also converted to decarboxylated AdoSeMet that functioned as a precursor of polyamine synthesis (spermidine and spermine) in a way similar to decarboxylated AdoMet. The byproduct MeSeAdo did not accumulate, and must thus have been further metabolized.

These findings indicate that SeMet is utilized by the cells similarly to methionine in the synthesis of adenosyl derivatives according to the metabolic pathways shown in Fig. 1. Further metabolism of SeHcy was not studied. Thus, conversion back to SeMet or catabolism to selenocysteine are hypothetical, and remain to be proven. Further studies are also needed to see if alternative metabolic reactions and pathways exist.

SeMet was shown to be cytotoxic to all tested cell lines in culture, and 50% growth-inhibition was brought about by 40–160 μ*M* SeMet addition to the culture medium containing 100 μ*M* methionine. That the toxic compound is either SeMet itself or its metabolite is supported by the finding that SeMet cytotoxicity was dependent on medium methionine

levels increasing with decreased methionine concentration. Such conditions favor increased uptake and metabolism of SeMet via the methionine metabolic pathways. Furthermore, the DL-form of SeMet was less toxic than the L-form. Only the L-form has a good substrate activity in AdoSeMet synthesis.

SeMet addition to the culture medium did not specifically block the synthesis of DNA, RNA, or protein. Also, ATP levels stayed normal. We did not observe any metabolic block in transmethylation or polyamine synthesis. It is tempting to speculate that toxicity may have been caused by selenocysteine formed via the transsulphuration pathway, especially because selenocysteine is several times more toxic than SeMet (E. O. Kajander, manuscript in preparation). However, the function of this pathway in SeMet catabolism has not been experimentally proved. Noteworthy, selenium and sulphur metabolism differ in their end products, and labeled SeMet has been shown to produce dimethylselenide and trimethylselenonium as final excreted products (*4*). Also, the activity of the transsulphuration pathway in cultured cells has been found to be low, and only a few percent of homocysteine was converted to cysteine (*20,21*). Further work is thus needed to elucidate the metabolism of selenohomocysteine and its role in SeMet toxicity. Finally, it must be underlined that SeMet administration resulted in a profound decrease in AdoMet level. This took place early (before a decrease in the number of viable cells occurred) and at SeMet levels, correlating well with the cytotoxicity. The fall in AdoMet was compensated by the formation of AdoSeMet. However, AdoMet is utilized in almost 100 reactions, some of which are vitally important. It remains to be clarified whether one or some of these reactions may not accept AdoSeMet as substrate, and thus, result in toxic effects.

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