THE NUTRITIONAL SIGNIFICANCE, METABOLISM AND TOXICOLOGY OF SELENOMETHIONINE

GERHARD NORBERT SCHRAUZER

Biological Trace Element Research Institute
2400 Boswell Rd., Suite 200
Chula Vista, CA 91914
USA

ADVANCES IN FOOD AND NUTRITION RESEARCH VOL 47
ISSN: 1043-4526
DOI: 10.1016/S1043-4526(03)47002-2

I. INTRODUCTION

Selenomethionine (SeMet), the selenium (Se-) analog of methionine (Met), was first suspected in the 1930s to be one of the toxic principles of seleniferous plants (Franke and Painter, 1938), although definite proof for its existence became possible only in 1947, after synthetic SeMet had become available (Klosterman and Painter, 1947). Chromatographic evidence also suggested SeMet to be present in seleniferous wheat protein extracts (Smith, 1949), which was confirmed by Peterson and Butler (1962), Butler and Peterson (1967) and Olson *et al.* (1970). Concurrent studies established that SeMet was also synthesized by certain marine algae (Shrift, 1954 a,b), *Escherichia coli* (Tuve and Williams, 1961), *Saccharomyces cerevisiae* (Blau, 1961), *Candida albicans* (Hedegaard *et al.*, 1963), and by rumen bacteria (Hidiroglou *et al.*, 1968). In 1962, ^{75}Se-labeled SeMet was introduced as a radioactive tracer and pancreatic radioimaging agent (Blau and Bender, 1962). The discovery of the nutritional essentiality of Se by Schwarz and Foltz (1957) created the need for an appropriate supplemental form of Se to prevent Se deficiency diseases in farm animals. Since synthetic SeMet was prohibitively expensive, sodium selenite and -selenate were approved for use as feed additives. These inorganic Se salts are readily absorbed and utilized for selenoenzyme synthesis but are less effective in raising blood Se levels than SeMet (Thomson and Stewart, 1974; Griffiths *et al.*, 1976; Robinson *et al.*, 1978; Thomson *et al.*, 1978; Van Rij *et al.*, 1979). To provide an economical food source of SeMet for use in human and animal nutrition, methods for the industrial production of Se-enriched yeast were developed by leading yeast manufacturers on initiative of Nutrition 21, a California nutritional supplement company. Presently, Se yeast is manufactured in many countries. In 1984, synthetic SeMet became available at a cost comparable to Se yeast on a *per se* basis. Methods of production of alternative food sources of SeMet such as high-Se wheat grains and of other Se-enriched crops were also devised (Gupta and MacLoed, 1998; Djusic *et al.*, 1998; Kivisaari, 1998; Whanger *et al.*, 1998).

II. PROPERTIES OF SELENOMETHIONINE

Selenomethionine [*2-amino-4-(methylseleno)-butanoic acid*], $CH_3-Se-CH_2CH_2CH(NH)_2-COOH$, ($C_5H_{11}NO_2Se$; C, 30.62%, H, 5.65%, N,

7.14%, O, 16.32%, Se, 40.26%, FW 196.11), Chemical Abstracts Number 2578-28-1, is a colorless compound with a musty odor, and crystallizes from aqueous acetone in the form of hexagonal crystals. SeMet exists in three forms. The L-form occurs naturally; the D-enantiomer and DL-mixture are obtained synthetically. Pure L-SeMet melts with decomposition at 274°C, DL-SeMet at 265°C, making it thermally somewhat less stable than its sulfur analog. SeMet is less soluble in water than Met due to the greater hydrophobicity of the CH_3-Se- as compared to the CH_3-S- group (Shepherd and Huber, 1969); at 30°C and pH 7.0. For example, saturated solutions of SeMet and Met are 0.108 and 0.386 M, respectively. In 2N HCl ($c = 0.02$), L-SeMet has an $[\alpha]_D$ of $+21.2°$ (Koch and Burchardt, 1993), its half-time of racemization of 19–20 days in aqueous solution at 100°C at pH 7.4 is similar to that of Met (Boehm and Bada, 1985; Mendez *et al.*, 1999). On acid hydrolysis, SeMet is significantly less stable than Met. In 6N HCl at 110°C, hydrolysis of SeMet was complete after 7 h, a treatment that leaves the sulfur analog Met still essentially unchanged (Chiao and Peterson, 1953). SeMet is also more oxygen-sensitive than Met, although the rates of oxidation and the role of promoters and inhibitors in oxidation remain to be determined. Methionine selenoxide, $CH_3-Se(O)-CH_2CH_2CH(NH)_2-COOH$, is formed on reaction of SeMet with hydrogen peroxide (Walter *et al.*, 1973). The oxidation also occurs efficiently with peroxynitrite (Assmann *et al.*, 1998). Whereas methionine sulfoxide is difficult to reduce to Met under physiological conditions, methionine selenoxide is easily converted back to SeMet on reaction with reducing agents such as glutathione (GSH), leading to the suggestion that SeMet acts catalytically as a cellular antioxidant (Walter *et al.*, 1973; Arteel *et al.*, 1999).

III. METHODS OF ANALYSIS

Pure SeMet may be distinguished from Met by its infrared spectrum (Shepherd and Huber, 1969). Identification of SeMet in protein hydrolysates is possible by means of standard amino acid analyzers (Sliwkowski, 1984). SeMet elutes near to, or with, leucine. Major factors affecting the resolution of these two amino acids are temperature and pH.

Identification of SeMet after being separated from other amino acids by means of paper chromatography and electrophoresis is facilitated by spraying spots with H_2O_2 or preferably by exposure to cyanogen bromide (Shepherd and Huber, 1969). Pre-derivatization of SeMet through reaction with *o*-benzoquinone facilitates separation and identification in the presence of Met. Both SeMet and Met react with *o*-benzoquinone at pH 1 to form phenolic sulfonium- or selenonium derivatives whose UV absorption spectra differ.

At pH 2, only Met reacts, allowing a distinction between the two compounds (Raju *et al.*, 1981). Reversed-phase high-performance liquid chromatography (RP-HPLC) involving post-column-derivatization with *o*-phthalaldehyde (OPA) was employed for SeMet determinations in high-Se yeast (Schrauzer, 1998a,b). For a description of state-of-the-art ion-exchange HPLC, ion-pair HPLC and RP-HPLC methods of analysis, see Bird *et al.* (1997a,b). For determinations by gas chromatography with inductively-coupled mass spectrometry (GC-ICP-MS), the carboxylic group of SeMet is esterified using propan-2-ol, followed by the acylation of the amino group with trifluoroacetic acid anhydride. Alternatively, the esterification and acylation of SeMet can be achieved in one step by a reaction with ethyl chloroformate–ethanol catalyzed by pyridine (Vasquez-Pelaez *et al.*, 2000). A method was described (Montes-Bayon *et al.*, 2001), allowing the detection of L- and D-SeMet by reversed-phase liquid chromatography following chiral derivatization with 1-fluoro-2,4,-dinitrophenyl-5-L-alanine amide (Marfey's Reagent), and inductively-coupled plasma spectrometry (ICP-MS) for detection.

Reliable enzymatic assays for SeMet are not available as specific SeMet metabolizing enzymes have not been identified and enzymes such as glutamine transaminase react with Met equally as well as with SeMet (Blazon *et al.*, 1994). However, with some enzymes reaction rates for SeMet and Met differ sufficiently to be of some use in SeMet analysis. For example, SeMet is a better substrate than Met for the α,γ-elimination by L-methionine γ-lyase of *Pseudomonas putida* (Esaki *et al.*, 1979). The adenosyl methionine transferase from rat liver reacts with L-SeMet at 51% of the rate with L-Met, and with the corresponding D-isomers at only 13 and 10% of the rate of L-Met (Pan and Tarver, 1967). Other adenosyl methionine transferases, such as that from yeast, react with SeMet more rapidly and with higher stereoselectivity than with Met, providing an indirect means for SeMet determination (Mudd and Cantoni, 1957; Sliwkowski, 1984; Uzar and Michaelis, 1994).

Indirect methods for SeMet determination utilize the facile reaction of SeMet with cyanogen bromide. The reaction of SeMet with BrCN yields CH_3SeCN, which can be measured either via gas chromatography (Zheng and Wu, 1988) or determined chemically (Zheng *et al.*, 1989). Applying this method to blood and grain, SeMet recoveries of 92.3–96.7% were achieved (Yang *et al.*, 1997a,b).

Use of appropriate methods of protein hydrolysis is of key importance irrespective of the method of SeMet determination used. Conditions must be chosen to achieve complete protein hydrolysis with minimal concomitant destruction of SeMet. In one study (Sliwkowski, 1984), SeMet recovery was 60–70% after heating the protein (thiolase from *C. kluyveri*) for 40 min at 155°C in 3 M mercaptoethanesulfonic acid. Recoveries of SeMet ranging

from 55 to 85% were obtained using 6N HCl under anaerobic conditions or pronase and prolidase for yeast or wheat protein hydrolysis (Beilstein and Whanger, 1986). SeMet recoveries of >90% are routinely accomplished with performic acid followed by the reduction of oxidized SeMet (Schrauzer, 1998a,b). The sample is first oxidized with performic acid followed by addition of HBr to remove excess performic acid. Performic acid oxidation converts SeMet to the selenium analog of a sulfone, which is more stable than SeMet during the subsequent 4 h hydrolysis/reduction step in 6N HCl and ascorbic acid addition at 145°C in a N_2-purged, sealed ampoule. SeMet is determined by RP-HPLC using post-column-derivatization with OPA. Primary nonaryl amines yield a strongly fluorescent derivative with OPA and 2-mercaptoethanol at pH 9 (borate buffer). Monitoring of the eluted peaks using UV prior to OPA treatment is used to augment the OPA-fluorescence detection.

IV. SYNTHESIS

Early syntheses of SeMet were tedious, non-stereospecific or limited to small-scale preparations. Klosterman and Painter (1947), for example, first reacted 5-(β-bromoethyl)-hydantoin with benzyl selenol to yield γ-benzylselenohomocysteine. The latter was converted to the sodium salt of DL-selenohomocysteine with sodium in liquid ammonia, and reacted with methyl iodide to yield DL-SeMet. Plieninger (1950) obtained DL-SeMet by the reaction of sodium selenomethyl mercaptide with α-amino-γ-butyrolactone in an inert solvent at 170°C. A synthesis of DL-SeMet from acrolein was also described (Zdansky, 1968). The first stereospecific synthesis of L-SeMet via esters of tosylated homoserine was reported by Pande *et al.* (1970).

In the newest method of synthesis, which allows the large-scale production of SeMet, L-Met is first converted to the methyl-methionium derivative, which is hydrolyzed to homoserine and converted to α-amino-γ-butyrolactone. The latter is reacted with HBr to 2-amino-4-bromobutanoic acid, from which L-SeMet is obtained on reaction with $LiSeCH_3$ (Koch and Burchardt, 1993; Krief *et al.*, 1994).

V. NATURAL OCCURRENCE AND BIOSYNTHESIS

A. PLANTS

Although Se is not known to be required for the growth of plants, most assimilate it when grown on seleniferous soils, or in Se-containing

hydroponic growth media. The degree of Se uptake is species-dependent. Primary Se-accumulators such as some species of *Astragalus*, *Stanleya*, and *Morinda* may, in high-Se regions, reach Se contents from hundreds to thousands of μg of Se/kg dry weight (Terry *et al.*, 2000). The tolerance of these plants to Se is attributed to their ability to convert Se into compounds that are not incorporated into the plant proteins and which, therefore, do not interfere with plant growth and metabolism. Detected Se compounds include SeMethylselenocysteine, selenocystathionine and γ-glutaminyl-SeMethylselenocysteine. Secondary Se-accumulators, such as cereals and forage crops, convert Se predominantly into SeMet. On normal soils, their Se contents range from 0.01 to 1 mg Se/kg dry weight, but levels toxic to foraging animals of up to 63 and 180 mg Se/kg dry weight were observed in wheat grown in high-Se areas of Canada and Columbia, respectively (Benavides and Silva, 1973). Selenate was sublethal to wheat seedlings when grown under experimental conditions in Keyport clay loam at levels of 15 to about 30 ppm Se, giving rise to a snow-white chlorosis, which, in sand cultures, is accompanied by a pink coloration (Hurd-Karrer, 1934). However, the toxicity of selenium to plants cannot be stated accurately as it is determined by the amount of sulfur available: whereas wheat seedlings in water cultures in the absence of added sulfate suffer distinct injury at selenium concentrations as low as 0.1 ppm, 18 ppm of Se as selenate was required to produce visible injury at sulfate-sulfur levels of 192 ppm (Hurd-Karrer, 1934). In mature secondary Se-accumulator plants, the SeMet is stored mainly in the grain and the roots, while smaller amounts are found in the stems and leaves (Beath, 1937). In plant tissue of the grassland legume species *Melilotus indica* L., for example, SeMet content increased with increasing soil Se concentrations until it accounted for more than 50% of the total selenoamino acids of the plant. In seleniferous corn, wheat and soybeans, SeMet contents ranged from 81 to 82% of the total Se (Yang *et al.*, 1997a,b). The biosynthesis of SeMet proceeds in analogy to that of Met via selenocysteine and selenocystathione according to Figure 1. The accumulation of SeMet in the protein of seleniferous plants is possible because $tRNA^{Met}$ does not discriminate between SeMet and its sulfur analog, Met; thus allowing its non-specific incorporation into proteins (Burnell, 1981; Eustice *et al.*, 1981). In contrast, replacement of cysteine (Cys) by selenocysteine (SeCys) would alter protein structure and does not occur to any significant extent. Moreover, since SeCys does not charge $tRNA^{Cys}$, the SeCys content of plants increases only moderately with increasing soil Se (from 5.07 to 22.02 mg/kg) and soon reaches a plateau. Other selenoamino acids, namely methylselenocysteine and γ-glutamyl-SeMethylselenocysteine, also remain at relatively low levels in secondary Se-accumulator plants, irrespective of the soil Se content (Guo and Wu, 1998). Plant-derived SeMet was detected in μg/kg levels in soil

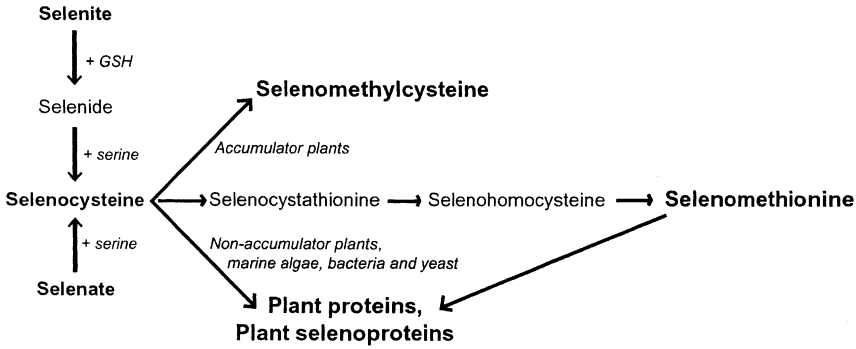

FIG. 1 Biosynthesis of selenomethionine; adapted from Marschner (1995); Schrauzer (2000).

extracts, indicating considerable resistance to further degradation, and suggesting that it could be an important source of plant-available Se (Abrams and Burau, 1989). Residual SeMet in soils could play a role as a dormancy-breaking agent for certain plants (Barros and De Paula-Freitas, 2001). The direct assimilation of SeMet by higher plants such as Indian mustard (*Brassica junctea*) has been demonstrated (Zayed *et al.*, 1998). SeMet in certain plants is methylated further to SeMethylselenonio-methionine (SeMethylSeMet) and cleaved to the volatile dimethylselenide (DMSe) (Figure 2). The latter may also be produced from dimethylselenoniopropionate (DMSeP), formed from SeMethylSeMet by decarboxylation, transamination and betaine aldehyde dehydrogenase.

The ability of plants to degrade SeMet to the DMSe is strongly species-dependent and represents a detoxification mechanism. However, Se volatilization may also result in losses of Se from food crops, aggravating Se deficiency in low-Se regions. Rice, broccoli and cabbage volatilized at the highest rates (200–350 μg Se/m^2 leaf area per day); and sugar beet, bean, lettuce and onion volatilized at lowest rates (Terry *et al.*, 1992).

B. MARINE PHYTOPLANKTON

Marine algae also convert Se predominantly into SeMet (Bottino *et al.*, 1984), Se uptake is dependent on the Se/S ratio in the medium and on the chemical form supplied. *Chlorella vulgaris* and *Chaetoceros calcitrans*, for example, take up Se(IV) preferentially over Se(VI). Some of the organoselenium compounds produced by these algae are not incorporated into cellular proteins but are extruded into the culture medium (Hu *et al.*, 1997). *Chlorella* also takes up SeMet; and its incorporation is antagonized by Met (Shrift, 1954a,b).

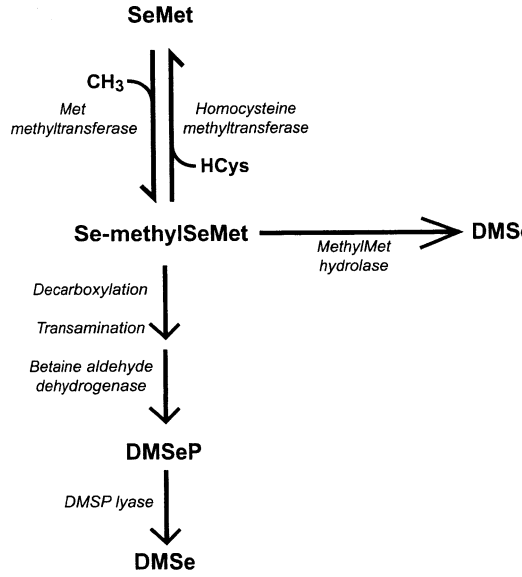

FIG. 2 Production of dimethylselenide (DMSe) from SeMet in plants via dimethylselenoniopropionate (DMSeP), adapted after Terry *et al.* (2000).

Spirulina incorporates Se when grown in Se-containing media. The resulting Se-rich spirulina contained the majority of the Se in the form of SeMet in two selenoproteins of 20–30 and 80 kDa. However, compared to the free SeMet and selenite, spirulina Se was less effective in restoring tissue Se levels and glutathione peroxidase (GSH-Px) activities (Cases *et al.*, 2001). The growth of spirulina in media containing 70–80 mg Se/l as SeO_2 was affected by the S:Se ratio and optimal at S : Se = 3 : 1. The Se content of biomass was 390 mg/kg at the Se concentration of 80 mg/l (as SeO_2) and the lowest sulfur concentration of 28 mg/l (Gradova *et al.*, 2001).

C. YEAST

S. cerevisiae converts inorganic Se to SeMet and incorporates it into the cellular protein in place of Met. The biosynthesis of SeMet proceeds via SeCys in analogy to that of Met, as was demonstrated in a study of a mutant strain of yeast requiring Met for growth due to a lack of homocysteine methyl transferase activity. When grown in Se-containing media, this strain produces SeCys, but no SeMet (Mason, 1994). While most of the SeCys is synthesized without involving Se-specific enzymes, recent studies indicate that some of the SeCys is also produced by a specific tRNA and incorporated into a 25 kDa

cytosolic protein. This protein migrates with the same velocity as the GSH-Px subunit isolated from rat liver and exhibits GSH-Px activity (Kyriakopoulos *et al.*, 1998). With currently available strains of *S. cerevisiae*, Se contents of up to 3000 ppm can be reached, at which level approximately half of the normally present cellular Met is replaced by SeMet. Commercial Se yeasts typically contain from 500 to 2000 ppm Se (Korhola *et al.*, 1986; Schrauzer, 1998a,b; Demirci *et al.*, 1999; Suhajda *et al.*, 2000). The Se is present predominantly (94 ± 5%) in the form of protein-bound SeMet (Schrauzer, 1998a,b). Other Se compounds include, in percentage of total Se: SeCys (0.5%), selenocystathionine (0.5%), methylselenocysteine (0.5%), γ-glutamyl-SeMethylselenocysteine (0.5%), Se-adenosyl-selenohomocysteine (2–5%) and inorganic Se (1%) (Schrauzer, 2000), bringing the Se balance in terms of identified Se compounds to 100 ± 5%. Previously reported SeMet contents of Se yeast were usually lower, largely, however, because of analytical problems and not because of differences in the actual SeMet contents. Lower than actual SeMet contents may result because of incomplete yeast protein hydrolysis and losses of SeMet due to hydrolysis and oxidation during sample digestion. The publication of these data led some authors to suspect that Se yeast was an unpredictable source of SeMet and was poorly characterized. Because Se yeast was used as a source of supplemental Se in the cancer prevention trial by Clark *et al.* (1996), the results of this trial were also called into question (Ip, 1998; Whanger, 2002). Analyses of water extracts of Se yeast (Bird *et al.*, 1997a,b) were initially interpreted similarly to suggest that the Se yeast contained only about 23% of the total Se in the form of SeMet, along with more than 20 unidentified Se-compounds (Ip, 1998; Whanger, 2002). However, water extracts of Se yeast analyzed contain only a fraction of the total Se (less than 10%), as most of the SeMet in Se yeast is protein-bound and insoluble in water. Accordingly, these results do not reflect the composition of Se yeast as a whole. Korhola *et al.* (1986) previously reported Se yeast to contain 50% of the total Se in the form of SeMet. However, even though the yeast in this study was hydrolyzed with acid under relatively vigorous conditions, the presence of unidentified Se-containing peaks in the chromatograms indicates that protein hydrolysis was incomplete. Beilstein and Whanger (1986) hydrolyzed Se yeast in 6N HCl in an inert atmosphere and achieved SeMet recoveries of 55–85%. Assuming an average recovery of 70%, they calculated the SeMet content of Se yeast to 93% of the total Se present, in agreement with the result of Schrauzer (1998a, b), who used performic acid for protein hydrolysis followed by the reduction of oxidized SeMet back to SeMet (see Section III). Oxidation of SeMet may occur during the enzymatic hydrolysis of Se yeast and may lower the SeMet recoveries. Thus, HPLC analysis of Se yeast after digestion with β-glucosidase revealed SeMet and what appeared to be SeMet oxide as the two

major Se compounds present (Larsen *et al.*, 2001). After Se yeast protein digestion with Protease XIV (Sigma, St. Louis), SeMet contents (85% of total Se) were obtained (Ip *et al.*, 2000). While Se yeast is generally a reliable source of SeMet, a cautionary note is necessary inasmuch as 'inorganic' Se yeast preparations containing only selenite or selenate are also marketed, often under the same name. The need for distinguishing between the two types has been emphasized (Schrauzer and McGinness, 1979; Gössler *et al.*, 1999; Schrauzer, 2001).

VI. SELENOMETHIONINE-CONTAINING PROTEINS AND ENZYMES

A. EFFECTS OF REPLACEMENT OF METHIONINE BY SELENOMETHIONINE

The replacement of Met by SeMet as a rule does not alter the protein structure, as evidenced from X-ray crystallographic studies of SeMet-substituted proteins. However, the presence of SeMet in place of Met may cause changes of contacts nearest to SeMet, altering the space group, as was recently demonstrated with oritidine 5′-monophosphate decarboxylase (Poulsen *et al.*, 2001). Since the CH_3-Se group of SeMet is more hydrophobic than the CH_3-S-moiety of Met, the kinetic reactivity of some enzymes changes if the replacement occurs in the vicinity of the active site. The SeMet-substituted thymidylate synthase of *E. coli*, for example, exhibited a 40% higher specific activity than the normal enzyme (Boles *et al.*, 1991). The SeMet-substituted phosphomannose isomerase from *C. albicans*, which normally contains four Met residues in the vicinity of the active site, had a four-fold higher K_M and a similarly increased inhibition constant for zinc ion, suggesting interference of substrate access due to the SeMet (Bernard *et al.*, 1995). In β-galactosidase of *E. coli*, replacement of more than half of the 150 Met residues by SeMet resulted in inactivity of this enzyme (Huber and Criddle, 1967). Compared to the normal enzyme, the thermal stability of the SeMet-substituted thymidylate synthase of *E. coli* was lowered eight-fold and its sensitivity to dissolved oxygen was significantly enhanced (Boles *et al.*, 1991).

The excessive replacement of Met by SeMet lowers the protein stability *in vitro*, but not necessarily *in vivo*. At the low levels of SeMet normally present in culture media, the substitution of Met by the more oxygen-sensitive SeMet is unlikely to affect the properties of enzymes in an adverse manner. Moreover, since methionine selenoxide is readily converted back to SeMet on reaction with biogenic thiols such as GSH, a mechanism of repair of this type of oxidative damage is available. The reversibility of SeMet oxidation in the

presence of GSH led to the suggestion that SeMet has the potential of being catalytically active as an antioxidant (Walter *et al.*, 1973), specifically against peroxynitrite (Arteel *et al.*, 1999). Since SeMet has radioprotective properties *in vitro* (Shimazu and Tappel, 1964), its presence in proteins could also directly or indirectly increase the resistance of cells to high-energy radiation UV light *in vivo*. Topical SeMet protects against UV light induced skin damage and skin cancer in mice (Burke *et al.*, 1992a,b).

B. SELENOMETHIONINE IN ORGANS AND TISSUES

In rats supplemented with Se in the form of sodium selenite, only SeCys, but no SeMet, was detected (Olson and Palmer, 1976). Since higher animals and man are unable to synthesize SeMet, any detectable amount in their organs and tissues must arise only from dietary sources. SeMet is incorporated into tissue proteins in place of Met (Martin and Hurlbut, 1976; Deagan *et al.*, 1987), especially in the skeletal muscles, the liver and the testes. The skeletal muscles are major Se-storage organs, accounting for about 46.9% of the total Se in the human body (Oster *et al.*, 1988). Se contents of skeletal muscle from Japanese adults were the highest (1700 ng/g), followed by those of Canadians (370 ng/g) and Americans (240 ng/g). Low-Se values (61 ng/g) were observed in skeletal muscle of New Zealand adults, reflecting their generally low-Se (and SeMet) intakes. Since erythrocytes incorporate SeMet mainly into hemoglobin (Waschulewski and Sunde, 1988), and in plasma SeMet is found primarily in the albumin fraction, Se levels in human blood also reflect the dietary SeMet intakes. In two samples of blood from Chinese men residing in a low-Se region, SeMet contents of 28.3 and 53.4 ng/g were observed, corresponding to 20–30% of the total Se (Yang *et al.*, 1997a,b). While the albumin of Chinese men with low-Se status contained 20% of the total Se, this percentage increased to 47 ± 5% in the albumin of men residing in a high-Se region. Their main dietary sources of SeMet were locally grown corn and rice and their average plasma Se level was 494 ng/ml (Beilstein *et al.*, 1992; Whanger and Butler, 1994). The same plasma Se concentration within experimental error (517 ± 84 ng/ml), and the identical percentage of plasma Se distribution, were observed in a study with Rhesus monkeys (*Macaca mulatta*) after they had been receiving 0.5 mg Se/l as SeMet in the drinking water during the first month, and 0.25 mg Se/l during the following 11 months (Butler *et al.*, 1990). In the livers, erythrocytes, hair and muscle of these animals, the Se concentrations were 3-, 5-, 7- and 11 times higher than in the corresponding organs of animals exposed to the equivalent amount of selenite Se. However, GSH-Px-activities in the plasma and in the erythrocytes were not different between the two treatment groups, re-affirming that SeMet is incorporated into protein to a higher extent than selenite. Similarly, in rats

fed a basal Se-deficient diet containing 2 ppm Se as SeMet, the level of Se in the muscle was 10 times that of the amount in rats receiving the equivalent amount of selenite or selenocysteine. In the liver, kidney and testes, the increase was 1.3–3.6-fold (Deagen *et al.*, 1987). In another study (Shearer, 1975), the muscle (thigh) of pregnant rats and of their pups contained approximately twice the amount of Se than the muscle of other animals when exposed to Se from the ninth day of pregnancy to the day of parturition to 0.2 ppm of SeMet in the drinking water (with some additional ^{75}Se–SeMet), than with the equivalent amount of selenite. The pup/mother ratio of Se contents of organs ranged mostly from 0.8 to 0.9, but was substantially higher for the teeth (molars: 7.71; incisors: 1.64) and bone (1.91). The high level of Se in the molars of the pups exposed to SeMet *in uteri* indicates that the incorporation of Se occurred endogenously during tooth development. In growing pigs, SeMet supplied in the form of Se yeast at the level of 0.3 ppm Se was about twice as effective as selenite in increasing the Se content of the loin muscle. SeMet in yeast also raised Se levels in porcine serum and liver significantly more than inorganic Se (Suomi and Alaviuhkola, 1992). Hair and fingernails of human subjects supplemented with SeMet contained a higher percentage of alkali-extractable Se than those receiving the same amount of Se in the form of selenate, indicating that SeMet is transported, deposited and metabolized differently than selenate (Whanger and Butler, 1994).

C. ORGAN DISTRIBUTION AFTER INJECTION

Injected ^{75}Se–SeMet produced high levels of radioactivity in the pancreas, the liver, the kidneys, in the stomach and the gastrointestinal mucosa. Lower but significant activities were observed in the salivary gland, the seminal vesicles, the mammary glands and bone marrow; the distribution pattern was similar to that observed with ^{35}S-methionine and ^{14}C-phenylalanine (Hansson and Jacobsson, 1966), indicating that SeMet is incorporated into organs with high rates of protein synthesis. Injection of the same amount of ^{75}Se in the forms of selenate or selenite resulted in much lower radioactivities in the respective organs as inorganic Se is converted only to SeCys in specific selenoproteins and is not incorporated into protein in place of Cys. The disappearance of intraperitoneally injected SeMet Se in tissues was slower than that of Se injected as selenite or selenate, especially from the brain. For example, in rats, ^{75}Se from ^{75}Se-sodium selenite disappeared from the cerebellum, the cerebral hemisphere, and the spinal cord very rapidly, 70% within 48 h, followed by a slower phase. The $t_{1/2}$ of Se disappearance was about 12 days for the cerebellum and the cerebral hemisphere, and 13 days from the spinal cord. Of an equivalent dose of ^{75}Se–SeMet, 20–30% disappeared during the first

48 h, the remainder with a $t_{1/2}$ of 25 ± 4 days from cerebellum and the cerebral hemisphere, and 29 ± 4 days from the spinal cord (Grønbaek and Thorlacius-Ussing, 1992). The high affinity of SeMet for the brain was also observed in rats after oral administration, suggesting that SeMet behaves like an active form of Se for incorporation in brain tissue. Selenomethionine was thus judged to serve as a better chemical form for Se-supplementation than either selenite or selenate (Wang *et al.*, 1992).

D. UPTAKE, RETENTION AND EXCRETION

Ingested SeMet is absorbed in the small intestine. The absorption occurs via the Na^+-dependent neutral amino acid transport system; and Met and other amino acids mutually inhibit SeMet absorption (Wolffram *et al.*, 1989; Vendeland *et al.*, 1994). The whole-body turnover rate of SeMet is slower than that of selenite-Se, indicating that the SeMet is incorporated into a long-term body pool. The reported average whole-body half-lives of SeMet and selenite in humans are 252 and 102 days, respectively, indicating that SeMet is extensively utilized and re-utilized (Patterson *et al.*, 1989; Swanson *et al.*, 1991). In a study with New Zealand women with low-Se status (Griffiths *et al.*, 1976), the intestinal absorption of a small oral dose of ^{75}Se–SeMet of approximately 20 mCi containing <2 μg Se was 95.5–97.3%. Approximately, 3% of this dose was excreted with the urine during the first day and 6–9% during the first 2 weeks. Urinary excretion of ^{75}Se continued to decrease gradually and at 44 weeks of the trial was about 0.08% dose/day. SeMet–Se was steadily incorporated into erythrocytes during a period of 8–12 weeks, with some radioactivity still persisting at 36 weeks. Plasma Se reached a maximum 3–4 h after administration and about 4–8 h sooner than after the administration of an equivalent dose of selenite. The urinary excretion rate of selenite was approximately twice that of SeMet and the fecal excretion of selenite Se was also faster initially, reaching 1.3–1.7 % of the dose in the first 14 days and dropping to less than 0.1% by weeks 10–19. After a single large oral dose of SeMet corresponding to 1 mg Se, 5–22% of dose was eliminated in 24 h in the urine and 3.5% in the feces. During the same period, 41–85% of the equivalent dose of selenite was excreted in the urine, and 11–13% with the feces. The total recovery of selenite reached 82–95% of the ingested dose, and 26% for SeMet (Thomson *et al.*, 1978). On continuing the supplementation for 11 weeks at 0.1 mg Se/day, SeMet caused a steady increase in blood Se from 0.08 to 0.18 μg Se/ml, the rate of increase being 0.009 μg Se/ml per week. The equivalent dose of selenite increased the blood Se more slowly until a plateau was reached after 7–8 weeks at 0.11 μg/ml. SeMet increased plasma Se more rapidly than erythrocyte Se, from 0.073 to 0.13 μg/ml, causing plasma Se to reach a higher concentration

than erythrocyte Se during the first two weeks of supplementation (Robinson *et al.*, 1978). SeMet is incorporated nonspecifically into albumin and becomes a part of the methionine pool (Burk *et al.*, 2001). Although the rates at which steady-state conditions are reached vary for different organs, protein turnover prevents accumulation of SeMet to toxic levels in the organism, causing blood Se levels to be linearly dependent on dietary Se intake over a wide range of intakes (Schrauzer and White, 1978; Yang *et al.*, 1989a). Similarly, hair, toenail and fingernail Se levels are proportional to the (long-term average) dietary Se intakes (Yang *et al.*, 1989a,b).

VII. METABOLISM OF SELENOMETHIONINE

A. ENZYMATIC DEGRADATION

SeMet is metabolized along with Met by the same enzymes and at similar rates until SeCys is formed. SeMet metabolism is rapid, as evidenced from the similar rates of appearance and disappearance of reactive Se metabolites in rat serum after oral administration of SeMet or selenite (Wang *et al.*, 1992). In contrast to Cys, which can be re-utilized for protein synthesis, SeCys is not appreciably incorporated into protein but further degraded in the liver to selenide. The latter is converted either to selenophosphate and used for the synthesis of SeCys-containing selenoproteins by specific tRNAs, or methylated to DMSe or the trimethylselenonium ion (TMSe) and excreted (Figure 3). Studies with rats and mice also demonstrated that SeMet is directly degraded by the action of α,γ-lyases in the liver to methylselenol (CH_3SeH), which is then converted to TMSe (Nakamuro *et al.*, 1997; Okuno *et al.*, 2001). The presence of the SeMet α,γ-lyases in the liver suggests that these serve primarily for SeMet detoxification. The adenoviral introduction of a bacterial α,γ-lyase gene to achieve this mode of SeMet degradation in tumor cells has been suggested as an approach to cancer gene therapy into tumor (Miki *et al.*, 2001).

Glutamine transaminase from bovine liver, one of the enzymes involved in methionine catabolism, utilizes SeMet as well as methionine (Blazon *et al.*, 1994). However, with some enzymes, differences in the reaction rates for SeMet and Met have been observed. For example, SeMet is a better substrate than Met for the α,γ-elimination by L-methionine γ-lyase of *Pseudomonas putida* (Esaki *et al.*, 1979). The adenosyltransferase from rat liver reacts with L(+)-SeMet at 51% of the rate with L(+)-Met, and with the corresponding D(−) isomers at only 13 and 10% of the rate of L-Met (Pan and Tarver, 1967). The adenosyl transferase from yeast, on the other hand, is more active with SeMet than with Met (Mudd and Cantoni, 1957). This enzyme produces the

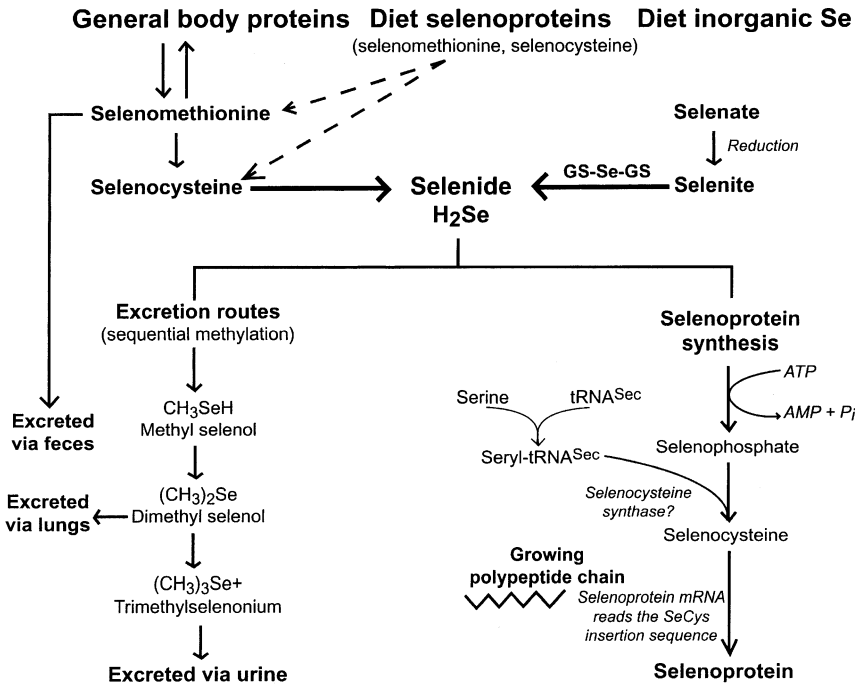

FIG. 3 Routes of selenium metabolism in animals; adapted from Jacques (2001), after Schrauzer (2000); Low and Berry (1996), and Daniels (1996).

adenosyl derivative of Met with low stereoselectivity due to the isomerization of Met during the incubation, while the corresponding formation of the Se-derivative occurs with >99% stereoselectivity (Uzar and Michaelis, 1994). SeMet neither blocks the metabolism of Met, nor the synthesis of DNA, RNA, or of proteins when added to the culture medium. Low SeMet levels (10 μM) replace Met and support cell growth in the absence of Met (Kajander *et al.*, 1991). In studies with human lymphoblast cells from subjects with trans-sulfuration defects, SeMet was poorly metabolized, as evidenced by the significantly higher levels required to induce GSH-Px activity, although these cells utilized selenite and selenocystine as well as normal cells (Beilstein and Whanger, 1992).

B. UTILIZATION FOR GLUTATHIONE PEROXIDASE SYNTHESIS

Glutathione peroxidases (GSH-Px) are enzymes catalyzing the reduction of hydrogen peroxide to water, and of lipid hydroperoxides to alcohols, with

GSH as the reductant. As GSH-Px was the first selenoenzyme to become known (Rotruck *et al.*, 1973), quantitative assessments of the bioavailabilities of different chemical forms of Se were initially based almost exclusively on the determination of the minimum amount of the Se compound required for the induction of GSH-Px in serum, erythrocytes, or liver. In interpreting the results of such determinations, it must be considered that they depend on the experimental conditions, the test animal and the diets chosen. In rats, for example, SeMet was as active as selenite in protecting against the development of liver necrosis. In chicks, SeMet was four times more active than selenite in protecting against pancreatic fibrosis, but was less effective than selenite in the prevention of exudative diathesis (Cantor *et al.*, 1975a; Mutanen, 1986). While liver GSH-Px activities induced by SeMet were slightly lower relative to selenite in the above-mentioned experiments, they were the same in the livers of nursing rat dams maintained on a low-Se diet to which 0.15 μg Se/g was added as either SeMet or as sodium selenite, and higher in the livers, kidneys and eyes of 14-day-old nursing pups from SeMet-supplemented dams than in pups of the same age from dams receiving Se as selenite. GSH-Px activities in the hearts were the same in both groups (Lane *et al.*, 1991). In Se-deficient heifers, supplementation with SeMet or Se yeast almost doubled the GSH-Px activity as compared to the equivalent amount of inorganic Se (Pehrson *et al.*, 1989). When ^{75}Se–SeMet was fed to ewes, the milk produced contained more Se available to the pre-ruminant lamb than milk from ewes receiving the equivalent amount of ^{75}Se in the form of selenite (Jenkins and Hidiroglou, 1971). The release of SeMet from body proteins during Se depletion causes SeMet-supplemented animals to maintain higher activities of selenoenzymes for longer periods than in animals supplemented with selenite. Thus, in SeMet- or Se yeast-supplemented mice, liver GSH-Px activities declined more slowly during the 7th to the 11th week of Se depletion than in mice that had previously received the equivalent amount of Se as sodium selenite (Spallholz and Rafferty, 1987). In nursing mothers, SeMet supplied as such or in Se yeast prevented the decline of plasma Se and GSH-Px activity, as well as the decline of Se in milk during lactation. In addition, significantly more Se appeared in the milk of mothers receiving SeMet than selenite (McGuire *et al.*, 1993; Alaejos and Romero, 1995). SeMet was detected in human milk (Michalke and Schramel, 1997).

C. FACTORS INFLUENCING TISSUE DEPOSITION AND BIOAVAILABILITY

In the living organism' structural and functional proteins are continuously synthesized and degraded within hours to days (Mitch and Goldberg, 1996).

In general, functional proteins and enzymes have higher turnover rates than structural proteins. The protein turnover rates are also dependent on age and are the highest during early development. Protein synthesis and degradation occur in separate compartments. Proteins are synthesized in the RNA-rich ribosomes, while the degradation of structural proteins occurs notably in pathological states such as starvation or following injury involving lysosome-localized proteases such as the cathepsins. Since some cells lack lysosomes and some structural proteins are too large to be internalized, additional means of degradation are available, which include cytosolic proteases such as the calpains and those in organelles, called proteasomes (Klasing, 1998). During stress, the activity of proteasomes increases to provide amino acids needed for the formation of immune defense cells and enzymes, including the Se-dependent GSH-Px and the thioredoxin reductases. During such times food intake is reduced, requiring reserves of amino acids and minerals to be utilized to a higher degree than in health or under stress-free conditions. It is at this point that the presence of SeMet in proteins becomes especially important since the SeMet released during protein catabolism provides a source of Se needed for the synthesis of GSH-Px and other selenoenzymes (Figure 4). The SeMet in the free amino acid pool is either incorporated into body proteins or degraded (Figure 4). Whereas the incorporation of SeMet into proteins is reversible, allowing SeMet to be re-used, its degradation is irreversible. The incorporation of SeMet into proteins and its degradation are dependent on the Met content of the diet. In one study, the Se-contents of muscle and erythrocytes of Met-deficient, SeMet-supplemented rats were shown to be significantly higher than those in SeMet-supplemented, Met-adequate animals (Sunde *et al.*, 1981). In another study (Butler *et al.*, 1989), a direct correlation was observed between the dietary Met and the percentage of Se associated with GSH-Px. In adult Chinese men with low-Se status with adequate but presumably limiting dietary Met intakes, supplemental Met increased the RBC GSH-Px activity (Luo *et al.*, 1987), indicating that less SeMet was deposited in protein, increasing the amount of Se available for GSH-Px synthesis.

On supplementing SeMet, GSH-Px activities reach a maximum value and then plateau, while tissue Se levels continue to rise in proportion to the dosage (Deagen *et al.*, 1987). The release of Se from SeMet also depends on vitamin B_6 status. In rats maintained on a feed supplemented with 0.25 mg Se/kg of diet in the form of SeMet, GSH-Px activities in erythrocytes, muscle and heart were lower in vitamin B_6-deficient than in vitamin B_6-supplemented animals (Yin *et al.*, 1996). Vitamin B_6 also significantly reduced lipid peroxide contents in tissues. These results indicate that dietary vitamin B_6 is involved in the metabolic breakdown of SeMet.

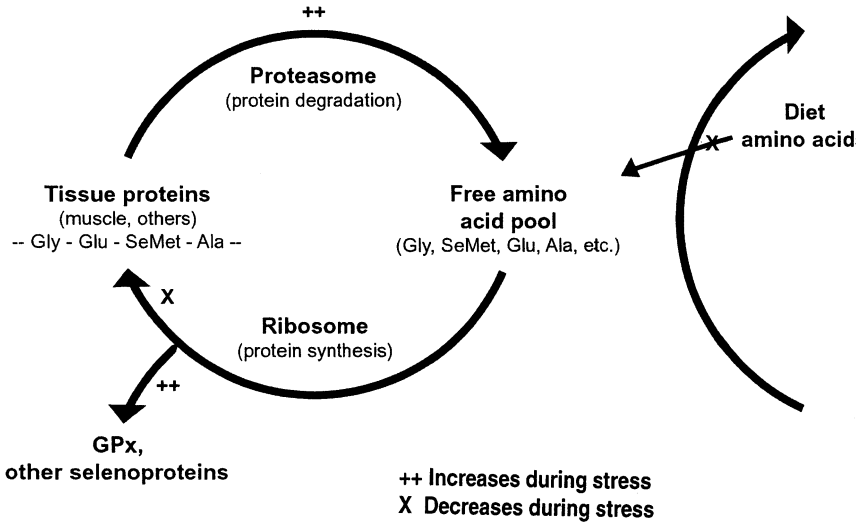

FIG. 4 Protein turnover releases stored selenomethionine (SeMet) to the free amino acid pool (Gly = glycine, Glu = glutamic acid, Ala = alanine). Adapted from Jacques, 2001.

D. SELENOMETHIONINE AND IMMUNE CELL FUNCTION

Selenium is well-known to be required for the maintenance of the functions of the immune system (McKenzie *et al.*, 2001). The immunomodulatory effects of Se depend on dosage and chemical form of Se administered. However, the required dosages of Se tend to be higher than the estimated minimum dietary requirements. In previous studies, supplementation of Se-replete subjects with 200 μg Se/day as sodium selenite resulted in increased T-lymphocyte proliferation and activity (Roy *et al.*, 1994). In gut failure patients on home parenteral nutrition, a daily parenteral dose of 200 μg Se in the form of L-SeMet elicited a significant increase of the antibody production against common mitogens (PWM and PHA) (Peretz *et al.*, 1994). There is also evidence that cells of the immune system discriminate between the different chemical forms of Se. Addition of SeMet to the medium of lymphocyte cultures, for example, produced no signs of cytotoxicity and increased the immunoglobulin G (IgG) production at levels of 0.5–2 μM, while selenite at equivalent concentrations decreased IgG production and exerted cytotoxic effects (Borella *et al.*, 1995). L-SeMet and sodium selenite also differ in their relative effectiveness in modulating GSH-Px activities of blood cells, as evidenced in a recent study with 45 healthy men and women from the UK

(Brown *et al.*, 2000). The enzyme activities measured include GSH-Px1, the cytosolic glutathione peroxidase; GSH-Px3, the extracellular glutathione peroxidase, and GSH-Px4, the phospholipid hydroperoxide GSH-Px. At a daily dose of 50 μg Se as SeMet, lymphocyte GSH-Px1 activities increased during the first two weeks of supplementation to an approximately 20% higher level than with the equivalent dose of selenite. The platelet GSH-Px1 activity also increased during the first two weeks of supplementation and more so with SeMet than with selenite, while no significant changes occurred in the placebo group. During the remaining two weeks of supplementation no further changes occurred in the SeMet and selenite groups, while a decline of the GSH-Px1 activities occurred in the placebo group. The granulocyte GSH-Px1 activity declined during the first 7 days of supplementation in the SeMet group, but subsequently increased during the following three weeks. In the selenite group, the granulocyte GSH-Px1 activities increased during the first week, declined to nearly the baseline value in the second week, only to increase again to the value measured after the first week in the following two weeks. The lymphocyte GSH-Px4 activities increased in the selenite-supplemented group during the first week of supplementation, and then declined during the following three weeks. In the SeMet-supplemented group, the lymphocyte GSH-Px4 activities increased slightly during the first week, declined during the second week, but after four weeks of supplementation, reached its maximum value. The granulocyte GSH-Px4 activities in the selenite group were also increased after the first week, lowered after the second, and increased again after the fourth week. With SeMet, the granulocyte GSH-Px4 activities increased during the first two weeks of supplementation and subsequently remained constant. Platelet GSH-Px4 activities increased in the selenite group during the first two weeks and subsequently declined. However, in the SeMet group, they remained essentially unchanged throughout the four weeks of supplementation. This was interpreted to suggest that immune cells take up and metabolize selenite-Se more rapidly than SeMet, and that the conversion of SeMet into reactive inorganic forms of Se may be an important regulator of Se bioavailability. The authors, furthermore, noted that the SeMet supplementation failed to increase plasma and erythrocyte GSH-Px activities in half of their subjects, which they attributed to possibly deficient dietary intakes of Met (Brown *et al.*, 2000).

VIII. SELENOMETHIONINE IN HUMAN SE SUPPLEMENTATION

The Se in SeMet was shown in numerous studies to be as bioavailable as naturally occuring nutritional sources of Se, and superior to inorganic Se in

improving the Se status of humans during lactation, in pregnancy and in infant nutrition (Schrauzer and White, 1978; Levander *et al.*, 1983; Kumpulainen *et al.*, 1985; Beilstein and Whanger, 1986; Korhola *et al.*, 1986; Spallholz and Rafferty, 1987; Vinson and Bose, 1987; Yang *et al.*, 1989a,b; McGuire *et al.*, 1993). Se yeast was used as the source of food-form Se in several human cancer prevention trials (Yu *et al.*, 1988; Clark *et al.*, 1996). The Clark study (Clark *et al.,* 1996) is especially relevant as it involved a daily administration of 200 μg of Se as Se yeast over a period of up to 7 years to conservatively treated nonmelanoma cancer patients. Whereas the Se-supplementation had no effect on skin cancer recurrence, it significantly reduced the incidences of cancers of prostate, lung, colon and rectum. At the dosage chosen, Se yeast was well tolerated. Mean serum Se levels at the steady state reached 190 ± 50 μg/l and did not result in accumulation to toxic levels. In a Chinese study (Yu *et al.*, 1988), a daily supplement of 200 μg Se as Se yeast provided to subjects at risk of developing primary liver cancer caused a substantial decline of liver cancer incidence within a year. Recently, synthetic SeMet was chosen as the source of Se in SELECT, the largest (prostate) cancer prevention study ever to be conducted (Klein *et al.*, 2001). Se yeast and SeMet were also tested in a number of small observational studies. In HIV-infected patients, supplemental SeMet (in yeast) produced symptomatic improvements (Olmsted *et al.*, 1988). Several studies are presently underway to further explore the effect of supplemental Se in HIV-infected patients (Baum *et al.*, 2001).

IX. SE YEAST AS AN ANIMAL FEED SUPPLEMENT

A. DAIRY COWS

Se yeast was shown to be superior to inorganic Se in improving the Se status of farm animals (Power, 1994; Lyons and Oldfield, 1996; Mahan and Kim, 1996; Hemken and Jacques, 1998; Ortman and Pehrson, 1999; Pavlata *et al.*, 2001). Since SeMet is synthesized by rumen bacteria from inorganic sources of Se (Hidiroglou *et al.*, 1968), selenite or selenate should improve the Se status of cattle equally as well as supplemental SeMet. However, the bacterial SeMet synthesis in the rumen is apparently either inefficient or the SeMet incorporated in the bacterial protein is poorly absorbed since, in dairy cows, SeMet supplied in yeast was more effective in increasing the concentration of Se in milk than added sodium selenite or -selenate. At feed Se contents of 0.21–0.31 mg/kg DM, milk Se reached 31.2 μg/l in the Se yeast group, but only 16.4 μg/l in the selenite and selenate groups (Ortman and Pehrson, 1999). Supplementation of dairy cows with Se yeast and vitamin E also

affected milk production, increasing it by 13.8% (Zemanova *et al.*, 2000). Previously, Conrad and Moxon (1979) showed that lactating cows transferred 19.8% of the Se in brewer's grains into the milk but only less than 4.8% of the Se supplied as selenite. Supplementation of nursing cows with Se yeast also improved the Se status of the calves more so than the equivalent amount of selenite (Mahan and Kim, 1996; Pehrson *et al.*, 1999), and improved the performance and meat quality in feedlot steers (Clyburn *et al.*, 2001).

Note added with proof: On Sept. 3, 2003, the selenium yeast product "Sel-Plex" (Alltech) was approved by the U.S. Food and Drug Administration as a source of selenium for beef and dairy cattle. See: Federal Register 68, No. 170.

B. PIGS

Addition of Se yeast to sow feed at 0.9 mg Se/sow/day starting three days prepartum increased the milk Se concentration 10-fold compared to controls (0.421 versus 0.046 mg Se/l). In 7-day-old piglets, the mean blood Se concentration was almost five times higher than in controls (0.221 versus 0.046 mg Se/l). In the experimental group, the weight gain of the piglets was higher and their mortality lower than in the controls. The Se yeast supplement also influenced the conception rate size and weight of the next litter (Kolacz *et al.*, 2001). Increasing the organic Se content of sow feed also has a vitamin E-sparing effect. Supplemental Se (0.2 ppm) in the form of Se yeast and vitamin E (20 IU) as DL-α-tocopherol acetate improved the litter size of sows; it also increased the number of piglets born alive compared to sows in the control group maintained on a diet with a Se content of only 0.1 ppm and a vitamin E content of 37 IU (Chen *et al.*, 2001). Se yeast (Sel-Plex™) fed to pigs at the FDA-approved dietary concentration of 0.3 ppm Se, alone or in combination with vitamin E (200 ppm) for 60 days prior to slaughter, decreased the metmyoglobin formation, especially in the psoas major muscle. In addition, the level of thiobarbituric acid-reactive substances in psoas major muscle was reduced after 7 days of carcass cool storage. A positive effect of Se plus vitamin E was also seen with respect to malondialdehyde formation in homogenates of longissimus dorsi muscle sampled after slaughter (Krska *et al.*, 2000).

C. POULTRY

SeMet as the source of Se restored appetite and liver GSH-Px activity in Se-deficient chicks more rapidly than selenite (Bunk and Combs, 1980) and was especially effective against pancreatic fibrosis (Cantor *et al.*, 1975b,c). In broiler chickens, Se yeast as the source of Se improved feathering rate in slow-feathering animals as compared to animals fed selenite or no Se. The Se

yeast also increased body weights and yields of leg and thigh meat and improved the efficiency of feed conversion. In addition, SeMet in yeast increased the stability of erythrocyte membranes, suggesting that this is the mechanism by which moisture retention is increased in the breast muscle from male broilers. Organic Se supplementation also improved the meat quality of broiler males and reduced drip loss from breast meat, while selenite Se appeared to increase drip loss. Furthermore broilers receiving yeast Se showed diminished levels of heat shock protein 70 (HSP 70) following challenge by enteropathogenic *E. coli*, or by acute heat stress (Edens, 2001). In June 2000, Se yeast (Sel-Plex™) was approved for broiler production in the United States (Anon, 2000) and approvals for other species are pending (Edens, 2001). Organic Se from yeast (Sel-Plex™) is readily transferred into the egg and is incorporated predominantly in egg albumin, while the Se from selenite and selenocysteine is deposited preferentially in the yolk (Latshaw and Osman, 1975; Latshaw and Biggert, 1981). Selenium supplementation has a sparing effect on vitamin E, causing the yolks of Se-supplemented hens to have significantly higher vitamin E contents (Surai, 2000), up to 27 mg per large egg compared to 0.70 mg in a regular egg (Slaugh, 2002). 'Designer eggs' with a high content of omega-3 fatty acids, obtained by feeding hens a 2% flax ration, require a higher vitamin E content than regular eggs to prevent the oxidation of the acids. Supplementing the diet of the layer hens with Se yeast (Sel-Plex™) prevented the oxidation of the omega-3 fatty acids and the loss of vitamin E of the eggs during a two-week storage period significantly more than the equivalent amount of Se as selenite. Supplementation of the feed with Se yeast also increased the duration of freshness of the eggs on storage (Wakebe, 1998). Addition of Se to the feed at the level of 0.3 ppm increased the albumin content of the eggs. Since SeMet is preferentially incorporated into albumin this may explain why the eggs from Se yeast-supplemented hens maintained better albumin quality on storage (Slaugh, 2002).

X. TOXICITY OF SELENOMETHIONINE

A. STORAGE AND HANDLING INSTRUCTIONS

Note: *Instructions were compiled from current Material Safety Data Sheets; for information only, not purported to be all-inclusive and to be used only as a guide.*

SeMet is classified as a very toxic substance. It should be stored in closed containers below 4°C in the freezer. SeMet should only be handled in a well-ventilated hood with rubber gloves, protective goggles and facial mask. To reduce the risk of accidental poisoning in the food and feed industry, pre-

mixes of SeMet rather than the pure substance should be used. First aid measures include immediately flushing the eyes with plenty of water for at least 15 min, occasionally lifting the upper and lower lids. Skin should be flushed with plenty of soap and water for at least 15 min while removing contaminated clothing and shoes. Upon accidental ingestion, do not induce vomiting. Allow the victim to rinse his mouth and then to drink 2–4 cupfuls of water and seek medical advice. On inhalation, remove victim from exposure to fresh air immediately. Exposure limits (NIOSH and OSHA), as Se: 0.2 mg/m^3.

B. ACUTE TOXICITY

As no human case of SeMet poisoning has been reported, all acute toxicity data for SeMet are derived from studies with animals. In general, the acute response to SeMet was delayed and not as severe as observed with selenite. Whereas sodium selenite, at 3.0 mg Se/kg, caused fatal selenium toxicosis in swine 2.5 h after intravenous injection, the equivalent amount of Se as SeMet was fatal after 14 h, with both compounds producing pulmonary edema as the main lesion (Herigstad *et al.*, 1973). The liver and kidney Se concentrations as determined by these authors reached 10.2 and 7.28 ppm in the animals injected with selenite, 11.3 and 10.8 ppm, in the animals given SeMet. The same authors showed that young pigs fed diets containing 60, 120 and 600 ppm of organic Se (SeMet) developed symptoms of acute toxicity after 125, 140 and 38 h on these feeds with a total of 49, 44 and 33 mg of Se being consumed. Liver Se levels reached 17.95, 34.5 and 27.4 ppm while kidney Se levels, 13,7, 11.1 and 12.8 ppm, respectively.

In rats, the LD_{50} of SeMet on intraperitoneal injection is 4.25 mg Se/kg body wt (Klug *et al.*, 1949). In mice, the LD_{50} of SeMet on intravenous and intracervical injection was determined to be 8.86 ± 1.38 and 5.24 ± 0.23 mg Se/kg, whereas the corresponding LD_{50} of sodium selenite was much lower, 2.28 ± 0.28 and 0.13 ± 0.04 mg Se/kg, respectively (Ammar and Couri, 1981).

C. CHRONIC TOXICITY

The chronic toxicity of SeMet is lower than that of sodium selenite, presumably because its incorporation into tissue proteins removes a portion from the circulatory system. The diminished toxicity of SeMet at dietary Se levels exceeding 5 ppm as compared to selenite was apparent in pigs in the grower–finisher period (Kim and Mahan, 2001a). In mature pigs, 5 ppm of Se as selenite as well as organic Se (SeMet in yeast) elicited similar toxic effects. However, in animals under conditions of high protein turnover such as during pregnancy, SeMet stored in tissues may re-enter circulation, resulting in increased toxicity as compared to selenite. High levels of organic Se also

affected gestation and parturition performance of sows more than the equivalent amount of Se as selenite. Especially at levels of >7 ppm Se, fewer piglets were born and pig weights at parturition and weaning were lower from sows fed organic Se than sodium selenite. This was attributed to the increased tissue turnover from the organic Se source (Kim and Mahan, 2001b). In addition, diminished placental transfer of inorganic as compared to organic Se could have been responsible, as evidenced by the lower tissue Se concentrations in the piglets from sows fed selenite compared to those sows fed SeMet. Nursing piglets from sows fed toxic levels of SeMet are at a further disadvantage compared to those from sows fed selenite due to the higher Se content of the milk from sows fed organic Se. In pigs fed SeMet as in Sel-Plex Se yeast, the higher Se content was evident already at the 0.3 ppm level. At the chronically toxic level of 7 ppm, the milk on day 14 reached 4.14 ppm compared to 0.76 ppm in the milk of animals receiving 7 ppm Se as sodium selenite. The greater deposition of SeMet into the organs of animals fed organic Se was evident at all levels between 0.3 and 10 ppm, and was particularly pronounced in the loin: at 10 ppm of SeMet Se in the feed, its Se content reached 5.33 ppm, compared to 0.23 ppm in the animals on selenite, corresponding to a factor of 23. Hair and hoofs of the animals fed SeMet contained 3.7 and 6.13 times more Se than of the animals on selenite, livers and kidneys 2–3 times more (Kim and Mahan, 2001b). In female mice, the injection of SeMet at 2.0 mg Se/kg body wt induced a transient decrease of the number of circulating leukocytes, which was more extensive and of longer duration than with selenate. The agranulocyte/granulocyte ratio also increased to a lesser degree with SeMet than with selenate (Hogan, 1998). The chronic toxicity of SeMet is lower than that of selenite. Rats fed high levels of Se (16 ppm) as SeMet (selenium yeast) for 8 weeks showed no signs of Se toxicity, while the same amount of Se as sodium selenite produced severe hepatotoxity, cardiotoxicity and splenomegaly (Spallholz and Rafferty, 1987). SeMet on oral administration did not produce toxic effects in rats at 0.5 and 1.0 mg SeMet (0.2 and 0.4 mg Se)/kg body wt/day in a 13-week study (NCI, 1993). However, in the same study, weight loss, decreased food consumption, liver abnormalities and toxic hepatitis occurred at 2, 3 and 4.5 mg SeMet (0.8, 1.2 and 1.8 mg Se)/kg body wt/day. Pancreatitis and atrophy or degeneration of pancreatic acini occurred at 0.8, 1.2 and 1.8 mg Se/kg body wt/day. At 1.2 and 1.8 mg Se/kg body wt/day, bile duct hyperplasia, telangiectasis, hemorrhage, necrosis, inflammation, vacuolar changes and brown pigment in hepatocytes, increased extramedullary hematopoiesis in livers and spleen were seen at 3 and 4.5 mg/kg body weight/day. Female rats were more sensitive than males with all of 10 females dead during 13 weeks, but only 1 male dead at 3 mg SeMet/kg body wt/day (1.2 mg Se/kg/day). In beagle dogs, no toxic effects of oral SeMet were

observed at 0.1 and 0.3 mg SeMet (0.04 and 0.12 mg Se)/kg body wt/day. At 1.0 mg SeMet (0.4 mg Se/kg body wt)/kg body wt/day, toxicity resulted in elevations of aspartate amino transferase (AST), glutamate pyruvate transaminase (GPT) and alanine amino transferase (ALT) activities; inflammation, telangiectasis, hemorrhage, vacuolar changes and brown pigment in hepatocytes, thymic atrophy, lymphocyte depletion in tonsils and intestine, gastrointestinal hemorrhage (NCI, 1993).

D. TOXICITY AND BIOPOTENCY OF THE D- AND L-ISOMERS

Additions of SeMet (D or L) at 6.25 mg/kg (corresponding to 2.5 mg Se/kg) over six weeks to the diet of rats produced no evidence of depressed growth or diminished survival. The same result was obtained with diets containing equivalent amounts of Se in the form of selenite or selenate (McAdam and Levander, 1987). However, severe growth depression and death of rats within 29 days occurred at 26 mg of D- or L-SeMet (10 mg Se) per kg of diet, as well as with the corresponding amounts of Se as selenite or selenate. In rats, D- and L-SeMet exhibited the same chronic toxicities at 13 mg of SeMet (5 mg Se) per kg of diet. At this level, the two isomers produced the same concentrations of Se in skeletal muscle, heart, liver and erythrocytes. Only the plasma Se levels were lower in the animals receiving L-SeMet (McAdam and Levander, 1987). Similarly, little difference in the retention of Se by liver and muscle tissue was seen when nutritional levels of D- or L-SeMet were fed to Se-depleted rats (McAdam and Levander, 1986). However, at the subtoxic level of 6.25 mg SeMet/kg diet, the Se concentrations were significantly higher in skeletal muscle, heart and liver in the animals receiving the D-isomer, indicating that the L-form is catabolized more rapidly under these conditions. Since adenosylation is the first step in the trans-sulfurization pathway of Met as well as of SeMet, this could cause the accumulation of D-SeMet in tissues and organs of species lacking an efficient mechanism of the conversion of D-Met into the L-form. In rats, which are known to utilize D-Met as well as L-Met, biopotency, acute toxicity and tissue retention of injected or dietary doses of both isomers of SeMet were about the same (McAdam *et al.*, 1985; McAdam and Levander, 1986,1987). In other species, the L-isomer was better utilized and more toxic than the D-isomer. In mallard ducklings, for example, Se as L-SeMet at 30 ppm in the diet was significantly more toxic than DL-SeMet, but concentrations of Se in the livers were about the same (Heinz *et al.*, 1996; Hoffman *et al.*, 1996). While normal mice metabolize both the isomers of SeMet equally well, more of the D-isomer was observed in the tumors of tumor-bearing mice. Within 48 h of administration, the *in vivo* uptake of D-SeMet was several times higher than that of the L-isomer in Ehrlich solid tumor, and in sarcoma 180 solid tumor. Since the uptake of both isomers was

about the same in the pancreas, this suggested the presence of a transport system specific for D-SeMet in tumor cells, in addition to a transport system common to both the D- and L- forms (Goto *et al.*, 1987). Furthermore experiments with cultured murine and human lymphoid cells demonstrated that the cells of the immune system also discriminate between the two enantiomeric forms of SeMet. Thus, DL-SeMet was only about half as cytotoxic than L-SeMet, and only L-SeMet was found to be a good substrate for adenosylmethionine synthetase and was effectively metabolized by trans-methylation reactions and in polyamine synthesis. The differential responses of the normal cells to L- and D-SeMet are thus attributable primarily to the initial steps of SeMet metabolism, which involve stereospecific trans-sulfurization enzymes preferentially utilizing L-SeMet. Differences in the activity of trans-methylation enzymes may also affect the cytotoxicity and the invasiveness behavior of tumor cells. For example, while pre-incubation with selenite resulted in a dose-dependent decrease in the ability of HeLa or NIH OVCAR-3 cells to invade a layer of a re-constituted basement membrane preparation, indicating diminished invasiveness, SeMet had no such effect (Gong and Frenkel, 1994).

E. MAXIMUM TOLERATED DOSE

In a 30-day trial with long-tailed female Macaques (*Macaca fascicularis*) receiving 0–600 μg of L-SeMet/kg day by nasogastric intubation, SeMet was well tolerated until the second to third week of the study at which time two animals given 600 μg/kg day died. One animal from the 300 μg/kg day group developed Se-induced hypothermia. Six animals in the 188 μg/kg day level or greater required supplements of fruit and *Sustagen* (Mead Johnson & Co, Evansville, IN), a powdered nutritional supplement for human consumption that is used to supply anorexic primates with necessary nutritional and caloric intake during periods when animals refuse their normal diet. With increasing SeMet dosage and duration of exposure, the incidence of anorexia, gastrointestinal distress, mucocutaneous toxicity, and frequency of reduced body temperature increased. A dose-dependent weight-loss was also observed. At the higher dosage, disturbances in the menstrual function were evident, and were accompanied by the absence of serum progesterone concentrations above 1.0 ng/ml, reduced luteal phase lengths, increased intermenstrual intervals, and lowered estrogen excretion. A "maximum tolerated dose" of 150 μg/kg day of L-SeMet for 30 days was identified based on mean body weight reduction, hypothermia, dermatitis, xerosis, cheilitis, disturbances in menstruation, and the need of dietary intervention to prevent death at doses of 188 μg/kg day or greater (Cukierski *et al.*, 1989). The same highest tolerated dose of L-SeMet (150 μg/kg day) on nasogastric intubation

was established with pregnant long-tailed Macaques (Hawkes *et al.*, 1992). In this study, the weight loss at a dosage of 300 μg Se/(kg day) of L-SeMet was three times greater than in the 150 μg Se/(kg day) group, and clear signs of Se toxicity (anorexia and vomiting) were apparent. The No-Effect Level was determined to the 375 μg SeMet/kg body wt day. At twice this dose, all animals developed anorexia, vomiting, and weight loss. Plasma Se levels increased in proportion to SeMet dosage only until day 21 and subsequently plateaued. Plasma GSH-Px activities increased in proportion to SeMet dosage and began to plateau after 20 days of administration. A steady state was reached at the two dosage levels when plasma Se concentrations had reached 1.91 and 3.86 μg/ml, respectively. However, erythrocyte and hair Se levels continued to increase until day 30 and for about two weeks after cessation of supplementation. Erythrocyte GSH-Px levels increased in proportion up to the SeMet dosage of 150 μg/kg body wt/day. At the toxic level of 300 μg/kg/day, erythrocyte GSH-Px activity was lower, and during the 30 days of dosing dropped to values only as high as in the 25 μg Se/kg body wt/day control group. However, in the 150- and 300 μg SeMet/kg body wt day animals, erythrocyte GSH-Px activity continued to increase for two weeks after cessation of supplementation, suggesting the release of SeMet from body storage organs. Erythrocyte Se, plasma Se and hair Se levels of >2.3 μg/ml, >2.8 μg/ml and >27 μg/g, respectively, were associated with increased weight loss due to Se toxicity. These values for Se toxicity are consistent with the cutoff values for humans subsisting on a predominantly vegetarian diet, as determined in a Chinese study (Liu and Li, 1987).

F. REFERENCE VALUES

From observations of populations residing in high-Se regions and evidence from supplementation studies, a *Reference Dose* (RfD) for selenium has recently been set to 350 μg Se/day for humans of 70 kg body weight periods (Patterson and Levander, 1997). This value corresponds to 5 μg Se/kg BW or five times the "Recommended Dietary Allowance" (RDA) for Se and represents the total intake of selenium from nutritional sources which are safe for indefinite periods, while still below the "maximal safe intake" of 450 μg/day, as defined by the British Committee on Medical Aspects of Food Policy (Department of Health, 1991). Another reference value is the "Tolerable Upper Intake Level," UL, representing "the highest level of daily selenium intake that is likely to pose no risk or adverse health effects to almost all individuals in the general population." The UL for selenium was set at 400 μg/day for adult, corresponding to one half of the No-Observed-Adverse-Effect-Level (NOAEL) of 800 μg/day. Although neither the RfD nor the UL specify

the chemical form of Se, both may be assumed to apply primarily to SeMet, the major nutritional form of Se. The RfD and UL thus set a safe limit for nutritional Se supplementation. In the cancer prevention trial of Clark *et al.* (1996), for example, the supplementary dose of 200μg Se/day, which consisted mainly of SeMet, increased the Se intakes of the study participants from 100–150 to 300–350 μg/day, causing the average plasma Se concentrations to increase, in 6–9 months, from 114 ± 22 to 190 ± 50μg/l. Selenium intakes of 450 μg/day or more were reached by the study participants of a Chinese liver cancer chemoprevention trial and produced no adverse effects (Yu *et al.*, 1988). In this study, supplementary Se at 400 μg Se/day was given, causing whole blood Se levels to reach 0.386 μg/ml after one year of supplementation. The Observed-NOAEL was provisionally estimated to 750–850 μg/day, based largely on observations with Chinese subjects residing in the high-Se areas. At 850 μg Se per day, blood Se levels reach 1 μg/ml, blood GSH concentrations were diminished and prothrombin time was reduced, with more significant signs of Se toxicosis appearing in one subject at 880 μg Se/day (Yang *et al.*, 1989a,b; Yang and Xia, 1995). However, in subjects from another area in China with Se intakes of 1457 ± 554 μg/day, selenosis was seen only occasionally (Yang and Zhou, 1994). From these observations an "Adverse Effect Level" (AEL), or "Individual Toxic Level", for dietary Se, of 1595 or 1600 μg/day was derived. This level of intake "seems capable of causing the development of chronic overt toxic selenosis after long-term intake" (Yang and Zhou, 1994; Yang and Xia, 1995). Earlier studies on subjects residing in high-Se regions of the United States suggested that mild, reversible signs of Se toxicity occur after 2400–3000 μg of Se/day for many months (NRC, 1976), suggesting that Se toxicity is influenced by diet' and particularly, the total protein intake; that high protein diets reduce Se toxicity is well known. Rats on a feed containing 35% casein and 35 ppm Se showed no signs of selenosis (Moxon and Rhian, 1943). The NOAELs and AELs for Se thus could be rendered more precise by relating them to the level of protein in the diet consumed. This could explain why adults receiving 1600 μg of nutritional Se per day for several months need not show overt signs of selenosis, while exhibiting blood Se levels of 1.61 μg/ml. Although the concentrations of Se in whole blood, plasma, hair, nails and urine are linearly dependent on the dietary Se-intakes (Schrauzer and White, 1978; Yang *et al.*, 1989a), these linear relationships are obeyed only if Se is ingested predominantly in the form of SeMet. If the ingested Se is in an inorganic form, blood and hair Se levels become unreliable indices of exposure and hence for diagnosis of selenosis, nail changes are the most significant signs (Yang *et al.*, 1989a,b).

XI. SELENIUM REQUIREMENTS AND RECOMMENDED DIETARY INTAKES

In 1980 the "Estimated Safe and Adequate Daily dietary Intake" (ESADDI) for adults and children above the age of 7 years was set at 50–200 μg Se/day (Food and Nutrition Board, 1980). Subsequently, based on Se balance studies, proposed 80 μg/day for men and 55 μg/day for women as safe and adequate Se intakes, corresponding to 1 μg Se/kg BW/day. In 1989, the RDA for selenium was lowered to 70 μg/day for adult men. This new intake recommendation was derived from the minimal amount of Se required for 100% saturation of the plasma GSH-Px activity. For adult Chinese males of 60 kg body weight, this amount had previously been determined to be 40 μg/day (Yang *et al.*, 1988). From this value, the RDA for North American males was calculated by taking into account their higher body weight (79 kg) and by increasing it further through multiplication with a safety factor. For adult women the RDA for selenium was maintained at 55 μg/day. For pregnant women, the RDA was set at 65 μg Se/day, for lactating women at 75 μg Se/day, assuming an average body weight of 63 kg.

In 1996, expert committees of the World Health Organization (WHO) derived basal and normative requirements for vitamins and minerals. The basal requirement was defined as "the intake needed to prevent pathologically relevant and clinically detectable signs of impaired functions attributable to inadequacy of the nutrient," and the normative intake as "the level of intake that serves to maintain a level of tissue storage or other reserve that is judged by the Expert Consultation to be desirable" (WHO, 1996). For selenium, the basal Se requirement was derived from the minimal amount needed to protect against Keshan disease, and was set at 21 μg/day for men and at 16 μg/day for women. The normative Se intakes were derived primarily under the assumption that two thirds of the maximum plasma GSH-Px activity affords sufficient antioxidant protection, corresponding to 40 and 30 μg/day for men and women, respectively. In the year 2000, the panel on dietary antioxidants and related compounds of the U.S. Food and Nutrition Board replaced the RDAs by "Dietary Reference Intakes" (DRIs), which for the most part numerically resembled the 1989 RDAs. All currently recommended Se intakes would be insufficient if selenium adequacy were assessed on immunological criteria such as antibody production or the cancer preventive effects of Se. In home-TPN patients receiving 50 μg Se per day, antibody production against common antigens was low compared to patients supplemented with 200 μg Se as SeMet (Peretz *et al.*, 1991). Similarly, 200 μg Se as SeMet in yeast was shown to be an effective dose of Se for cancer prevention, see Section F before. Obviously, all intake recommendations depend on the definitions used to derive them; they are not to be

considered final and must be re-evaluated and revised periodically. Specifically for Se, revisions of current intake recommendations are likely to be forthcoming in the light of rapidly advancing research and the growing recognition of its protective effects at supranutritional intakes.

XII. SUMMARY AND CONCLUSIONS

SeMet is a naturally occurring toxic amino acid but at the same time represents the major nutritional source of selenium for higher animals and humans. The ability of SeMet to be incorporated into the body proteins in place of Met furthermore provides a means of reversible Se storage in organs and tissues. This property is not shared by any other naturally occurring selenoamino acid and thus could be associated with a specific physiological function of SeMet. Since higher animals cannot synthesize SeMet, yet from it all needed forms of Se are produced, SeMet meets the criteria of an essential amino acid. Accordingly, SeMet, or enriched food sources thereof, are appropriate forms of Se for human nutritional Se supplementation. However, while SeMet or Se yeast are already widely used in over-the-counter nutritional supplements, infant formulas and parenteral feeding mixtures still contain Se in the form of sodium selenate or sodium selenite, even though these are not the normal nutritional forms of Se. In animal nutrition, these inorganic selenium salts are increasingly replaced by food sources of SeMet such as Se yeast. Synthetic SeMet could also be employed as a feed additive, but its regulatory status is as yet undetermined. The optimal nutritional levels of SeMet for different animal species still need to be determined. The expectation is that lower additions to feedstock of equivalent levels of SeMet will suffice to achieve adequacy than currently approved maximum levels of Se in the form of inorganic Se salts.

REFERENCES

Abrams, M.M. and Burau, R.G. 1989. Fractionation of selenium and detection of selenomethionine in a soil extract. *Commun. Soil Sci. Plant Anal.* **20**, 221–237.

Alaejos, M.S. and Romero, C.D. 1995. Selenium in human lactation. *Nutr. Rev.* **53**, 159–166.

Ammar, E.M. and Couri, D. 1981. Acute toxicity of sodium selenite and selenomethionine in mice after ICV or IV administration. *Neurotoxicology* **2**, 383–386.

Anonymous, 2000. Food additives permitted in feed and drinking water of animals; selenium yeast. *Fed. Register* **56**(109), 35823–35824.

Arteel, G.E., Gavin, E., Bribiva, K., and Sies, H. 1999. Protection against peroxynitrite. *FEBS Lett.* **445**(2,3), 226–230.

Assmann, A., Briviba, K., and Sies, H. 1998. Reduction of methionine selenoxide to selenomethionine by glutathione. *Arch. Biochem. Biophys.* **349**, 201–203.

Barros, R.S. and De Paula-Freitas, A.W. 2001. Selenomethionine as a dormancy-breaking agent in seeds of *Stylosanthes humilis*. *Acta Physiol. Plant.* **23**(3), 279–284.

Baum, M.K., Campa, A., Miguez-Burbano, M.J., Burbano, X., and Shor-Posner, G. 2001. Role of selenium in HIV/AIDS. *In* "Selenium—Its Molecular Biology and Role in Human Health" (D.L. Hatfield, ed.), pp. 247–255. Kluwer Academic Publishers, Boston/Dordrecht/London.

Beath, O.A. 1937. The occurrence of selenium and seleniferous vegetation in Wyoming. II. Seleniferous vegetation in Wyoming. *Wyoming Agric. Exp. Stn Bull.* **221**, 29–64.

Beilstein, M.A. and Whanger, P.D. 1986. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. *J. Nutr.* **116**, 1701–1710.

Beilstein, M.A. and Whanger, P.D. 1992. Selenium metabolism and glutathionine peroxidase activity in cultured human lymphoblasts. *Biol. Trace Elem. Res.* **35**, 105–118.

Beilstein, M.A., Whanger, P.D., and Yang, G.O. 1992. Chemical forms of corn and rice grown in a high selenium area of China. *Biomed. Environ. Sci.* **4**, 392–398.

Benavides, S.T., Silva, F. 1973. Mojica Selenosis, Instituto Geografico 'Augustin Codazzi', Bogota, Columbia (1965), see Jaffé W.G., Qualitas Planarum, Plant Food for Human Nutrition. XXIII: 191.

Bernard, A.R., Wells, T.N., Cleasby, A., Borlat, F., Payton, M.A., and Proudfoot, A.E. 1995. Selenomethionine labelling of phosphomannose isomerase changes its kinetic properties. *Eur. J. Biochem.* **230**, 111–118.

Bird, S.M., Ge, H., Uden, P.C., Tyson, J.F., Block, E., and Donoyer, E. 1997a. High performance liquid chromatography of selenoamino acids and organo selenium compounds. Speciation by inductively coupled plasma mass spectrometry. *J. Chromatogr.* **789**, 349–359.

Bird, S.M., Uden, P.C., Tyson, J.F., Block, E., and Denier, A. 1997b. Speculation of and compounds in selenium-enriched yeast using high-performance liquid chromatography-inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **12**, 785–788.

Blau, M. 1961. Biosynthesis of (^{75}Se)selenomethionine and (^{75}Se)selenocystine. *Biochem. Biophys. Acta* **49**, 389–390.

Blau, M. and Bender, M.A. 1962. (^{75}Se)selenomethionine for visualization of the pancreas by isotope scanning. *Radiology* **78**, 974–979.

Blazon, C., Coccia, R., Pensa, B., Cini, C., and De Marco, C. 1994. Selenomethionine as substrate for glutamine transaminase. *Biochem. Mol. Biol. Int.* **32**, 79–86.

Boehm, M.F. and Bada, J.L. 1985. The racemization rate of selenomethionine and methionine in yeast at 100°C and neutral pH. *Anal. Biochem.* **145**, 273–276.

Boles, J.O., Censors, R.J., Weir, M.S., Doom, J.D., Villa franca, J.E., and Dunlap, R.B. 1991. Purification and characterization of selenomethionyl thymidylate synthase from *Escherichia coli*; comparison with the wild-type enzyme. *Biochemistry* **30**(46), 11073–11080.

Borella, P., Bargellini, A., and Medici, C.I. 1995. Chemical form of selenium greatly affects metal uptake and responses by cultured human lymphocytes. *Biol. Trace Elem. Res.* **51**, 43–54.

Bottino, N.R., Banks, C.H., Irgolic, K.J., Micks, P., Wheeler, A.E., and Zingaro, R.A. 1984. Selenium containing amino acids and proteins in marine algae. *Phytochemistry* **23**, 2445–2452.

Brown, K.M., Pickard, K., Nicol, F., Beckett, G.J., Duthie, G.G., and Arthur, J.R. 2000. Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphocytes, granulocytes, platelets and erythrocytes. *Clin. Sci.* **98**, 593–599.

Bunk, M.J. and Combs, J.F. 1980. Effect of selenium on appetite in the selenium-deficient chick. *J. Nutr.* **110**, 743–749.

Burk, R.F., Hill, K.E., and Motley, A.K. 2001. Plasma selenium in specific and non-specific forms. *BioFactors* **14**(1–4), 107–114.

Burke, K.E., Bedford, R.G., Combs, G.F. Jr., French, I.W., and Skeffington, D.R. 1992a. The effect of topical L-selenomethionine on minimal erythema dose of ultraviolet irradiation in humans. *Photodermatol. Photoimmunol. Photomed.* **9**, 52–57.

Burke, K.E., Combs, G.F., Gross, E.G., Bhuyan, K.C., Gross, E.G., and Abu Lideh, H. 1992b. The effects of topical and oral L-selenomethionine on pigmentation and skin cancer induced by ultraviolet irradiation. *Nutr. Cancer* **17**, 123–137.

Burnell, J.N. 1981. Methionyl-tRNA synthetase from *Phaseolus aureus*: purification and properties. *Plant Physiol.* **67**, 316–324.

Butler, G.W. and Peterson, P.J. 1967. Uptake and metabolism of inorganic selenium-75 by *Spirondela oligorhizza*. *Aust. J. Biol. Sci.* **20**, 77–86.

Butler, J.A., Beilstein, M.A., and Whanger, P.D. 1989. Influence of dietary methionine on the metabolism of selenomethionine in rats. *J. Nutr.* **119**, 1001–1009.

Butler, J.A., Whanger, P.D., Kaneps, A.J., and Patton, N.M. 1990. Metabolism of selenite and selenomethionine in the rhesus monkey. *J. Nutr.* **120**, 751–759.

Cantor, A.H., Langevin, M.L., Moguchi, T., and Scott, M.L. 1975a. Efficacy of selenium in selenium compounds and feedstuffs for prevention of pancreatic fibrosis in chicks. *J. Nutr.* **105**, 106–111.

Cantor, A.H., Scott, M.L., and Noguchi, T. 1975b. Biological availability of selenium in feedstuffs and selenium compounds for prevention of pancreatic fibrosis in chicks. *J. Nutr.* **105**, 106–111.

Cantor, A.H., Langevin, M.L., Noguchi, T., and Scott, M.L. 1975c. Efficacy of selenium in selenium compounds in feedstuffs and selenium compounds for prevention of exudative diathesis in chicks. *J. Nutr.* **105**, 106–111.

Cases, J., Vacchina, V., Napolitano, A., Caporiccio, B., Besancon, P., Lobinski, R., and Rouanet, J.-M. 2001. Selenium from selenium-rich spirulina is less bioavailable than from sodium selenite and selenomethionine in selenium-deficient rats. *J. Nutr.* **131**(9), 2343–2350.

Chen, S.C., Chen, S.D., Wang, G.S., Liou, J.J., and Chang, F.C. 2001. On-farm trials of organic selenium to improve sow herd fertility. *Taiwan Tangye Yanjiuso Yanjiu Huibao* **170**, 45–54.

Chiao, J.S. and Peterson, W.H. 1953. Yeasts: methionine and cystine contents. *Agric. Food Chem.* **1**(15), 1005–1008.

Clark, L.C., Combs, G.F., Turnbull, B.W., Slate, E.H., Chalker, D.K., Chow, J., Davis, L.S., Glover, R.A., Graham, G.F., Gross, E.G., Krongrad, A., Lesher, J.L. Jr., Park, H.K., Sanders, B.B. Jr., Smith, C.L., and Taylor, J.R. 1996. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. *J. Am. Med. Assoc.* **276**, 1957–1963.

Clyburn, B.S., Richardson, C.R., Montgomery, J.L., Pollard, G.V., Herring, A.D., and Miller, M.F. 2001. Effect of selenium source and vitamin E level on performance and meat quality of feedlot steers. *In* "Science and Technology in the Feed Industry" (T.P. Lyons and K.A. Jacques, eds), pp. 377–392. Nottingham University Press, Nottingham, UK.

Conrad, H.R. and Moxon, A.L. 1979. Transfer of dietary selenium to milk. *J. Dairy Sci.* **62**, 404–411.

Cukierski, M.J., Willhite, C.C., Lasley, B.L., Hendrie, T.A., Book, S.A., Cox, D.N., and Hendricks, A.G. 1989. 30-day oral toxicity study of L-selenomethionine in female long-tailed macaques (*Macaca fascicularis*). *Fundam. Appl. Toxicol.* **13**, 26–39.

Daniels, L.A. 1996. Selenium metabolism and bioavailability. *Biol. Trace Elem. Res.* **54**, 185–199.

Deagen, J.T., Butler, J.A., Beilstein, M.A., and Whanger, P.D. 1987. Effects of dietary selenite, selenocysteine and selenomethionine on selenocysteine lyase and glutathione peroxidase activities in rat tissues. *J. Nutr.* **117**, 91–98.

Demirci, A., Pometto, A.L., and Cox, D.J. 1999. Enhanced organically bound selenium yeast production by fed-batch fermentation. *J. Agric. Food Chem.* **47**, 2496–2500.

Department of Health, UK. 1991. Dietary reference values for food energy and nutrients for the United Kingdom (Report on Health and Social Subjects, No. 41). London, Her Majesty's Stationery Office.

Djusic, I.S., Djermanovic, V., and Milovac, M. 1998. Foliar supplementation with selenium the efficient way to increase Se content in wheat grain, its products and human consumers. *In* "Proc. 6th Internat. Symp. Uses of Selenium and Tellurium" (Y. Palmieri, ed.), pp. 129–134, Scottsdale, AZ. Grimbergen.

Edens, F.W. 2001. Involvement of Sel-Plex in physiological stability and performance of broiler chickens. *In* "Science and Technology in the Feed Industry" (T.P. Lyons and K.A. Jacques, eds), pp. 349–376. Nottingham University Press, Nottingham, UK.

Esaki, N., Tanaka, H., Uemura, S., Suzuki, T., and Soda, K. 1979. Catalytic action of L-methionine gamma-lyase on selenomethionine and selenols. *Biochemistry* **18**, 407–410.

Eustice, D.C., Krull, F.J., and Shrift, A. 1981. Selenium toxicity: aminoacylation and peptide bond formation with selenomethionine. *Plant Physiol.* **67**, 1054–1058.

Food and Nutrition Board, 1980. Committee on Dietary Allowances. *In* Recommended Dietary Allowances, 9th Revised Edition, National Academy Press, Washington D.C., p. 185.

Franke, K.W. and Painter, E.P. 1938. A study of the toxicity and selenium content of the seleniferous diets: with statistical consideration. *Cereal Chem.* **15**, 1–24.

Gössler, W., Zheng, J., and Kosmus, W. 1999. Speziation von Selen in Supplementierungspräparaten. *In* "Spurenelemente—Speziationsanalyse, Supplementierung und Therapie mit Spurenelementen" (D. Meißner, ed.), Wissenschaftliche Verlagsgesellschaft mbH Stuttgart, pp. 61–64.

Gong, Y. and Frenkel, G.D. 1994. Effect of selenite on tumor cell invasiveness. *Cancer Lett.* **78**, 195–199.

Goto, R., Unno, K., Okada, S., and Tamemasa, O. 1987. Tumor accumulation of D-selenomethionine-75Se in tumor-bearing mice. *J. Pharmacobio-Dyn.* **10**(9), 456–461.

Gradova, N.B., Sukhinina, E.A., Babusenko, E.S., Gusarova, N.A., and Baum, I.F. 2001. Growth of Spirulina on selenium containing media. *Biotekhnologia* **2001**(5), 40–44.

Griffiths, N.M., Stewart, R.D.H., and Robinson, M.F. 1976. The metabolism of (^{75}Se)selenomethionine in four women. *Br. J. Nutr.* **35**, 119–127.

Grønbaek, H. and Thorlacius-Ussing, O. 1992. Selenium in the central nervous system of rats exposed to 75-Se selenomethionine and sodium selenite. *Biol. Trace Elem. Res.* **35**, 119–127.

Guo, X. and Wu, L. 1998. Distribution of free seleno-amino acids in plant tissue of *Melilotus indica* L. grown in selenium-laden soils. *Ecotoxicol. Environ. Saf.* **39**(3), 207–214.

Gupta, U.C. and MacLoed, J.A. 1998. Selenium enrichment of crops and selenium in ground water following selenium fertilization. *In* "Proc. 6th Internat. Symp. Uses of Selenium and Tellurium" (Y. Palmieri, ed.), pp. 119–126, Scottsdale, AZ. Grimbergen.

Hansson, E. and Jacobsson, S.-O. 1966. Uptake of [^{75}Se]selenomethionine in the tissues of the mouse studied by whole-body autoradiography. *Biochim. Biophys. Acta* **115**, 285–293.

Hawkes, W.C., Willhite, C.C., Craig, K.A., Omaye, S.T., Cox, D.N., Choy, W.N., and Hendrickx, A.G. 1992. Effects of excess selenomethionine on selenium status indicators in pregnant long-tailed Macaques (*Macaca fascicularis*). *Biol. Trace Elem. Res.* **35**, 281–297.

Hedegaard, J., Falcone, G., and Calabro, S. 1963. Incorporation de selenium dans des analogues des acides amines soufr dans *Candida albicans*. *C. R. Soc. Biol.* **157**, 280–285.

Heinz, G.H., Hoffman, D.J., and LeCaptain, L.J. 1996. Toxicity of seleno-L-methionine, seleno-D,L-selenomethionine, high selenium wheat, and selenized wheat to mallard ducklings. *Arch. Environ. Contam. Toxicol.* **31**(1), 93–99.

Hemken, R.W. and Jacques, K.A. 1998. Bioavailability of selenium yeast (Sel-Plex) vs sodium selenite for dairy cattle. Proceedings of the 6th International Symposium on the Uses of Selenium and Tellurium, Selenium-Tellurium Development Association, May 10–12, 1998, Scottsdale, AZ, USA. pp 161–162.

Herigstad, R.R., Whitehair, C.K., and Olson, O.E. 1973. Inorganic and organic selenium toxicosis in young swine: comparison of pathologic changes with those in swine with vitamin E-selenium deficiency. *Am. J. Vet. Res.* **34**, 1227–1238.

Hidiroglou, M., Heaney, D.P., and Jenkins, K.J. 1968. Metabolism of inorganic selenium in rumen bacteria. *Can. J. Physiol. Pharmacol.* **46**(2), 229–232.

Hoffman, D.J., Heinz, G.H., LeCaptain, L.J., Eiseman, J.D., and Pendleton, G.W. 1996. Toxicity and oxidative stress of different forms of organic selenium and dietary protein in mallard ducklings. *Arch. Environ. Contam. Toxicol.* **31**(1), 120–127.

Hogan, G.R. 1998. Selenate- and selenomethionine-induced leukopenia in ICR female mice. *J. Toxicol. Environ. Health, Part A* **53**, 113–119.

Hu, M., Yang, Y., Martin, J.M., Yin, K., and Harrison, P.J. 1997. Preferential uptake of Se(IV) over Se(VI) and the production of dissolved organic Se by marine. *Mar. Environ. Res.* **44**, 225–231.

Huber, R.E. and Criddle, R.S. 1967. The isolation and properties of beta-galactosidase from *Escherichia coli* grown in sodium selenate. *Biochim. Biophys. Acta* **141**, 587–599.

Hurd-Karrer, A.M. 1934. Selenium injury to wheat plants and its inhibition by sulphur. *J. Agric. Res.* **49**, 343–357.

Ip, C. 1998. Lessons from basic research in selenium and cancer prevention. *J. Nutr.* **128**, 1845–1854.

Ip, C., Birringer, M., Block, E., Kotrebai, M., Tyson, J.F., Uden, P.C., and Lisk, D.J. 2000. Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. *J. Agric. Food Chem.* **48**, 2062–2070.

Jacques, K.A. 2001. Selenium metabolism in animals: the relationship between dietary selenium form and physiological response. *In* "Science and Technology in the Feed Industry" (T.P. Lyons and K.A. Jacques, eds), pp. 319–348. Nottingham University Press, Nottingham, UK.

Jenkins, K.J. and Hidiroglou, M. 1971. Transmission of selenium as selenite and as selenomethionine from ewe to lamb via milk using selenium-75. *Can. J. Anim. Sci.* **51**, 389–403.

Kajander, E.O., Harvima, R.J., Eloranta, T.O., Martikainen, H., Kantola, M., Kaerenlampi, S.O., and Akerman, K. 1991. Metabolism, cellular actions, and cytotoxicity of selenomethionine in cultured cells. *Biol. Trace Elem. Res.* **28**(1), 57–68.

Kim, Y.Y. and Mahan, D.C. 2001. Comparative effects of high dietary levels of organic and inorganic selenium on selenium toxicity of growing-finishing pigs. *J. Anim. Sci.* **79**, 942–948.

Kim, Y.Y. and Mahan, D.C. 2001. Prolonged feeding of high dietary levels of organic and inorganic selenium to gilts from 25 kg body weight through one parity. *J. Anim. Sci.* **79**, 956–966.

Kivisaari, S. 1998. Selenium in NPK fertilizers: twelve years experience in its effects on crop production. *In* "Proc. 6th Internat. Symp. Uses of Selenium and Tellurium" (Y. Palmieri, ed.), pp. 113–118, Scottsdale, AZ. Grimbergen.

Klasing, K.C. 1998. Comparative Avian Nutrition. CAB International, New York, NY.

Klein, E.A., Thompson, I.M., Lippman, S.M., Goodman, P.J., Albanes, D., Taylor, P.R., and Coltman, C. 2001. SELECT: the next prostate cancer prevention trial. Selenum and Vitamin E Cancer Prevention Trial. *J. Urol.* **166**(4), 1311–1315.

Klosterman, H.J. and Painter, E.P. 1947. An improved synthesis of the selenium analog of methionine and homocystine. *J. Am. Chem. Soc.* **69**, 2009–2010.

Klug, H.L., Petersen, D.F., and Moxon, A.L. 1949. The toxicity of selenium analogues of cystine and methionine. *Proc. S. D. Acad. Sci.* **28**, 117.

Koch, T. and Burchardt, O. 1993. Synthesis of L(+)-selenomethionine. *Synthesis*, 1065–1067.

Kolacz, R., Dobrzanski, Z., and Grudnik, T. 2001. Effect of selenium yeast feed supplement on selenium level in sow milk and piglet blood serum and on production performance. *Folia Univ. Agric. Stetinensis* **224**, 77–81.

Korhola, M., Vainio, A., and Edelmann, K. 1986. Selenium yeast. *Ann. Clin. Res.* **18**, 65–68.

Krief, A., Trabelsi, M., and Shastri, M. 1994. Efficient synthesis of (D,L)- and D-selenomethionine. *In* "Proc. of STDA's Fifth Internatl. Symposium" (S.C. Carapella, J.E. Oldfield, and Y. Palmieri, eds), pp. 107–109, Brussels, May 8–10.

Krska, P., Lahucky, R., Palanska, O., Kuchenmeister, U., Kuhn, G., Demo, P., and Bahelka, I. 2000. Effect of organic selenium on fattening ability and meat quality in pigs. *J. Farm Anim. Sci.* **33**, 153–159.

Kumpulainen, J., Salmenperä, L., Simes, M.A., Koivistonen, P., and Perheentupa, J. 1985. Selenium status of exclusively breast-fed infants as influenced by maternal organic and inorganic selenium supplementation. *Am. J. Clin. Nutr.* **42**, 829–835.

Kyriakopoulos, A., Rothlein, D., Pfeifer, H., Gessner, H., and Behne, D. 1998. Selenium-containing compounds in backerUs yeast (*Saccharomyces cerevisiae*). *In* "Proc. Mengen-Spurenelemente Arbeitstagung, 18th" (M. Anke, ed.), pp. 740–745. Harald Schubert Verlag, Leipzig, Germany.

Lane, H.W., Strength, R., Johnson, J., and White, M. 1991. Effect of chemical form of selenium on tissue glutathione peroxidase activity in developing rats. *J. Nutr.* **121**, 80–86.

Larsen, E.H., Hansen, M., Fan, T., and Vahl, M. 2001. Speciation of selenoamino acids, selenonium ions and inorganic selenium ion by exchange HPLC with mass spectrometric detection and its application to yeast and algae. *J. Anal. At. Spectrom.* **16**(12), 1403–1408.

Latshaw, J.D. and Biggert, M.D. 1981. Incorporation of selenium into egg proteins after feeding selenomethionine or sodium selenite. *Poult. Sci.* **60**, 1309–1313.

Latshaw, J.D. and Osman, M. 1975. Distribution of selenium in egg white and yolk after feeding natural and synthetic selenium compounds. *Poult. Sci.* **54**(4), 1244–1252.

Levander, O.A., Alfthan, G., Arvilommi, H., Gref, C.G., Huttunen, J.K., Kataja, M., Koivistoinen, P., and Pikkarainen, J. 1983. Bioavailability of selenium in Finnish men as assessed by glutathione peroxidase activity and other blood parameters. *Am. J. Clin. Nutr.* **37**, 887–897.

Liu, B.-S. and Li, S.-S. 1987. Endemic selenosis, and fluorosis. *In* "Selenium in Biology and Medicine, Part B" (G.F. Combs, J.E. Spallholz, O.A. Levander, and J.E. Oldfield, eds), pp. 708–711. AVI Van Nostrand Reinhold Company, New York.

Low, S.C. and Berry, M.J. 1996. Knowing when not to stop. Selenocysteine incorporation in eukaryotes. *TIBS* **21**, 203–208.

Luo, X.M., Wei, H.J., Yang, C.L., Xing, J., Liu, X., Qiao, C.H., Feng, Y.M., Liu, Y.X., and Wu, O. 1987. Bioavailability of selenium to residents in a low-selenium area of China. *In* "Selenium in Biology and Medicine, Part A" (G.F. Combs, J.E. Spallholz, O.A. Levander, and J.E. Oldfield, eds), pp. 436–444. AVI Van Nostrand Reinhold Company, New York.

Lyons, T.P. and Oldfield, J.E. 1996. The case for organic selenium. *Bull. Selenium-Tellurium Dev. Assoc.* **June Issue**, 1–3 (Grimbergen Belgium).

Mahan, D.C. and Kim, Y.Y. 1996. Effect of inorganic and organic selenium at two dietary levels on reproductive performance and tissue selenium concentrations in first parity gilts and their progeny. *J. Anim. Sci.* **74**, 2711–2718.

Marschner, H. 1995. "Mineral Nutrition of Higher Plants". Academic Press/Harcourt Brace & Company, London/New York.

Martin, J.L. and Hurlbut, J.A. 1976. Tissue selenium levels and growth responses of mice fed selenomethionine, Se-methylselenocysteine or sodium selenite. *Phosphorus and Sulfur* **1**, 295–300.

Mason, A.C. 1994. Selenium incorporation into selenocysteine in yeast proteins. *FASEB J.* **8**(4), A541.

McAdam, P.A. and Levander, O.A. 1986. Comparative biopotency of dietary D- or L-selenomethionine (SeMet) with or without added methionine (Met) in rats. *Am. J. Clin. Nutr.* **43**, 672.

McAdam, P.A. and Levander, O.A. 1987. Chronic toxicity of dietary selenium fed to rats as D- or L-selenomethionine, selenite or selenate. *Nutr. Res.* **7**, 601–610.

McAdam, P.A., Wood, L.E., and Levander, O.A. 1985. Comparative acute toxicity and nutritional biopotency of injected doses of D- versus L-selenomethionine (SeMet) in rats. *Am. J. Clin. Nutr.* **41**, 864.

McGuire, M.K., Burgert, S.L., Milner, J.A., Glass, L., Kummer, R., Deering, R., Boucek, R., and Picciano, M.F. 1993. Selenium status of lactating women is affected by the form of selenium consumed. *Am. J. Clin. Nutr.* **58**, 649–652.

McKenzie, R.C., Rafferty, T., Beckett, G.J., and Arthur, J.R. 2001. Effect of selenium on immunity and aging. *In* "Selenium in Molecular Biology and Role in Human Health" (D.L. Hatfield, ed.), pp. 257–272. Kluwer Academic Publishers, Boston/Dordrecht/London.

Mendez, S.P., Bayon, M.M., Gonzalez, E.B., and Sanz-Medel, A. 1999. Selenomethionine chiral speciation in yeast and parenteral solutions by chiral phase capillary gas chromatography-ICP-MS. *J. Anal. At. Spectrom.* **14**(9), 1333–1337.

Michalke, B. and Schramel, P. 1997. Selenium speciation in human milk with special respect to quality control. *Biol. Trace Elem. Res.* **59**, 45–56.

Miki, K., Xu, M., Gupta, A., Ba, Y., Tan, Y., Al-Refaie, W., Bouvet, M., Makuuchi, M., Moossa, A.R., and Hoffman, R.M. 2001. Methioninase cancer gene therapy with selenomethionineas suicide prodrug substrate. *Cancer Res.* **61**(18), 6805–6810.

Mitch, M.E. and Goldberg, A.L. 1996. Mechanisms of muscle of wasting. *N. Engl. J. Med.* **335**(25), 1897–1905.

Montes-Bayon, M., B'Hymer, C., Ponce de Leon, C., and Caruso, J.A. 2001. Resolution of seleno-amino acid optical isomers using chiral derivatization and inductively coupled plasma mass spectrometric (ICP-MS) detection. *J. Anal. At. Spectrom.* **16**(9), 945–950.

Moxon, A.L. and Rhian, M. 1943. Selenium poisoning. *Physiol. Rev.* **23**, 305–327.

Mudd, S.H. and Cantoni, G.L. 1957. Selenomethionine in enzymatic transmethylations. *Nature* **180**, 1052.

Mutanen, M. 1986. Bioavailability of selenium. *Ann. Clin. Res.* **18**, 48–54.

Nakamuro, K., Nakanishi, K., Okuno, T., Hasegawa, T., and Sayato, Y. 1997. Comparison of methylated selenium metabolites in rats after oral administration of various selenium compounds. *Jpn. J. Toxicol. Environ. Health* **43**, 182–189.

NCI. 1993. "Proc. Workshop on Selenium Compounds in Cancer Chemo Prevention Trials." Division of Cancer Prevention and Control, National Cancer Institute, National Institutes of Health, Bethesda, MD, September 20–21.

National Research Council. 1976. Selenium. *In* "Medical and Biologic Effects of Environmental Pollutants", pp. 28–35. National Academy of Sciences, Washington, DC.

Okuno, T., Kubota, T., Kuroda, T., Ueno, H., and Nakamuro, K. 2001. Contribution of an enzymatic α, γ-elimination reaction in detoxification pathway of selenomethionine in mouse liver. *Toxicol. Appl. Pharmacol.* **176**(1), 18–23.

Olmsted, L., Schrauzer, G.N., Flores-Arce, M., and Dowd, J. 1988. Selenium supplementation of symptomatic immunodeficiency virus infected patients. *Biol. Trace Elem. Res.* **15**, 167–177.

Olson, O.E. and Palmer, I.S. 1976. Selenoamino acids in tissues of rats administered inorganic selenium. *Metabolism* **25**, 299.

Olson, O.E., Novacek, E.J., Whitehead, E.I., and Palmer, I.S. 1970. Investigations on selenium in wheat. *Phytochemistry* **9**, 1181–1188.

Ortman, K. and Pehrson, B. 1999. Effect of selenate as a feed supplement to dairy cows in comparison to selenite and selenium yeast. *J. Anim. Sci.* **77**(12), 3365–3370.

Oster, O., Schmiedel, G., and Prellwitz, W. 1988. The organ distribution of selenium in German adults. *Biol. Trace Elem. Res.* **15**, 23–45.

Pan, F. and Tarver, H. 1967. Comparative studies on methionine, selenomethionine and the ethyl analogues as substrates for methionine adenosyltransferase from rat liver. *Arch. Biochem. Biophys.* **119**, 429–434.

Pande, C.S., Rudick, J., and Walter, R. 1970. Synthesis and application in peptide chemistry of amino acids possessing an optically active selenohomocysteine skeleton. *J. Org. Chem.* **35**, 1440–1445.

Patterson, B. and Levander, O.A. 1997. Naturally occurring selenium compounds in cancer chemoprevention trials: a workshop summary. *Cancer Epidemiol. Biomark. Prevent.* **6**, 63–69.

Patterson, B., Levander, O., Helzsouer, K., McAdam, P., Lewis, S., Taylor, P., Veillon, C., and Zech, L.A. 1989. Human selenite metabolism. A kinetic model. *Am. J. Physiol.* **257**, R556–R567.

Pavlata, L., Illek, J., and Pechova, A. 2001. Blood and tissue selenium concentrations in calves treated with inorganic or organic selenium compounds. *Acta Vet. Brno* **70**(1), 19–26.

Pehrson, B., Knutsson, M., and Gyllensward, M. 1989. Glutathione peroxidase activity in heifers fed diets supplemented with organic and inorganic selenium compounds. *Swed. J. Agric. Res.* **19**, 53–59.

Pehrson, B., Ortman, K., Madjid, N., and Trafikowska, U. 1999. The influence of dietary selenium as selenium yeast or sodium selenite on the concentration of selenium in the milk of suckler cows and the selenium status of their calves. *J. Anim. Sci.* **77**(12), 3371–3376.

Peretz, A., Nève, J., Duchateau, J., Siderova, V., Huygen, K., Framaey, J.-P., and Carpentier, Y.A. 1994. Effects of selenium supplementation on immune parameters in gut failure patients on home parenteral nutrition. *Nutrition* **7**, 215–221.

Peterson, P.J. and Butler, G.W. 1962. The uptake and assimilation of selenite by higher plants. *Aust. J. Biol. Sci.* **15**, 126–146.

Plieninger, H. 1950. The cleavage of γ-butyrolactone and α-amino-γ-butyrolactone with sodium methylmercaptide or selenide. A synthesis of methionine. *Chem. Ber.* **83**, 265–268.

Poulsen, J.-C.N., Harris, P., Jensen, K.F., and Larsen, S. 2001. Selenomethionine substitution of oritidine-5′-monophosphate decarboxylase causes a change in crystal contacts and space group. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **D57**(9), 1251–1259.

Power, R.F. 1994. Selenium enriched yeast: form and applications. "Proc. STDA 5th Internatl. Symp.", pp. 89–94156. May 8–10. Brussels, STDA, Brussels.

Raju, K., Gopinath, R., and Gupta, M.N. 1981. Reaction of selenomethionine with *o*-benzoquinone. *Anal. Biochem.* **118**, 227–230.

Robinson, M.F., Rea, H.M., Friend, G.M., Stewart, R.D.H., Snow, P.C., and Thomson, C.D. 1978. On supplementing the selenium intake of New Zealanders. 2. Prolonged metabolic experiments with daily supplements of selenomethionine, selenite and fish. *Br. J. Nutr.* **39**, 589–600.

Rotruck, J.T., Pope, A.E., Ganther, H.E., Swanson, A.B., Hafeman, D.G., and Hoekstra, W.G. 1973. Selenium: biochemical role as a component of glutathionine peroxidase. *Science* Washington **179**, 588.

Roy, M., Kiremidjian-Schumacher, L., Wishe, H., Cohen, M.W., and Stotzky, G. 1994. Supplementation with selenium and human immune cell functions. I. Effect on lymphocyte proliferation and interleukin 2 receptor expression. *Biol. Trace Elem. Res.* **41**, 103–114.

Schrauzer, G.N. 1998a. Selenomethionine and selenium yeast: appropriate forms of selenium for use in infant formulas and nutritional supplements. *J. Med. Foods* **1**, 201–206.

Schrauzer, G.N. 1998b. Characterization of selenium yeasts for nutritional selenium supplementation. *In* "Proc. Sixth Internatl. Symp. on the Uses of Selenium and Tellurium" (Y. Palmieri, ed.), pp. 77–79, May 10–12. Scottsdale, AZ.

Schrauzer, G.N. 2000. Selenomethionine: a review of its nutritional significance, metabolism and toxicity. *J. Nutr.* **130**, 1653–1656.

Schrauzer, G.N. 2001. Nutritional selenium supplements: product types, quality, and safety. *J. Am. Coll. Nutr.* **20**, 1–4.

Schrauzer, G.N. and White, D.A. 1978. Selenium in human nutrition: dietary intakes and effects of supplementation. *Bioinorg. Chem.* **8**, 303–318.

Schrauzer, G.N. and McGinness, J.E. 1979. Observations on human selenium supplementation. *Trace Subst. Environ. Health XIII*, 64–67.

Schwarz, K. and Foltz, C.M. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.* **79**, 3292–3293.

Shearer, T.E. 1975. Developmental and post developmental uptake of dietary organic and inorganic selenium into the molar teeth of rats. *J. Nutr.* **105**, 338–347.

Shepherd, L. and Huber, R.E. 1969. Some chemical and biochemical properties of selenomethionine. *Can. J. Biochem.* **47**, 877–881.

Shimazu, F. and Tappel, A.L. 1964. Selenoamino acids decrease radiation damage to amino acids and proteins. *Science* **143**, 369–371.

Shrift, A. 1954a. Sulfur-selenium antagonism. I. Antimetabolite action of selenate on the growth of *Chlorella vulgaris*. *Am. J. Bot.* **41**, 351–358.

Shrift, A. 1954b. Sulfur-selenium antagonism II. Antimetabolite action of selenomethionine on the growth of *Chlorella vulgaris*. *Am. J. Bot.* **41**, 345–352.

Slaugh, B. 2002. Nutrition-specific eggs: an even more perfect food. *In* "Nutritional Biotechnology in the Feed and Food Industries" (T.P. Lyons and K.A. Jacques, eds), pp. 289–293. University of Nottingham Press, Nottingham, UK.

Sliwkowski, M.X. 1984. Characterization of selenomethionine in proteins. *Methods Enzymol.* **107**, 620–623.

Smith, A.L. 1949. Separation of the selenium compounds in seleniferous plant protein hydrolysates by paper partition chromatography. Thesis in partial fulfillment of the requirements for the degree of Master of Science, South Dakota State College of Agriculture and Mechanic Arts, August 1949.

Spallholz, J.E. and Rafferty, A. 1987. Nutritional, chemical and toxicological evaluation of high-selenium yeast. *In* "Selenium in Biology and Medicine, Part A" (G.F. Combs, J.E. Spallholz, O.A. Levander, and J.E. Oldfield, eds), pp. 516–529. AVI Van Nostrand Reinhold Company, New York.

Suhajda, A., Hegoczki, J., Janzso, B., Pais, I., and Vereczkey, G. 2000. Preparation of selenium yeast I. Preparation of selenium-enriched *Saccharomyces cerevisiae*. *J. Trace Elem. Med. Biol.* **14**(1), 43–47.

Sunde, R.A., Gutzke, G.E., and Hoekstra, W.G. 1981. Effect of dietary methionine on the biopotency of selenite and selenomethionine in the rat. *J. Nutr.* **111**, 76–86.

Suomi, K. and Alaviuhkola, T. 1992. Response to organic and inorganic selenium on the performance and blood Se content of growing pigs. *Agric. Sci. Finl.* **1**, 211–215.

Surai, P.F. 2000. Organic selenium: benefits to animals and humans, a biochemist's view. *In* "Biotechnology in the Feed Industry, Proceedings of Alltech's 16th Annual Symposium" (T.P. Lyons and K.A. Jacques, eds), pp. 205–260. Nottingham University Press,, Nottingham, UK.

Swanson, C.A., Patterson, B.H., Levander, O.A., Vellon, C., Taylor, P., Helzsouer, K., McAdam, P.A., and Zech, L.A. 1991. Human [^{74}Se]selenomethionine metabolism: a kinetic model. *Am. J. Clin. Nutr.* **54**, 917–926.

Terry, N., Carlson, C., Raab, T.K., and Zayed, A.M. 1992. Rates of selenium volatilization among crop species. *J. Environ. Qual.* **21**, 342–344.

Terry, N., Zayed, A.M., de Souza, M.P., and Tarun, A.S. 2000. Selenium in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51**, 401–432.

Thomson, C.D. and Stewart, R.D.H. 1974. The metabolism of (^{75}Se)selenite in young women. *Brit. J. Nutr.* **32**, 47–57.

Thomson, C.D., Burton, C.E., and Robinson, M.F. 1978. On supplementing the selenium intake of New Zealanders. I. Short experiments with large doses of selenite or selenomethionine. *Br. J. Nutr.* **39**, 579–587.

Tuve, T. and Williams, H.H. 1961. Metabolism of selenium by *Escherichia coli*: biosynthesis of selenomethionine. *J. Biol. Chem.* **236**, 597–601.

Uzar, H.G. and Michaelis, J. 1994. The metabolism of L-methionine/L-selenomethionine: NMR-studies. *GIT Fachz. Lab.* **38**(12), 1214 (see also pp. 1216–1218).

Van Rij, A.M., Thomson, C.D., McKenzie, J.M., and Robinson, M.F. 1979. Selenium deficiency in total parenteral nutrition. *Am. J. Clin. Nutr.* **32**, 2076–2085.

Vasquez-Pelaez, M., Montes Bayon, M., Garcia Alonso, J.I., and Sanz-Medel, A. 2000. A comparison of different derivatization approaches for the determination of selenomethionine by GS-ICP-MS. *J. Anal. At. Spectrom.* **15**(9), 1217–1222.

Vendeland, S.C., Deagen, J.T., Butler, J.A., and Whanger, P.D. 1994. Uptake of selenite, selenomethionine and selenate by brush border membrane vesicles isolated from rat small intestine. *BioMetals* **7**, 305–312.

Vinson, J.A. and Bose, P. 1987. *In* "Selenium in Biology and Medicine, Part B" (G.F. Combs, J.E. Spallholz, O.A. Levander, and J.E. Oldfield, eds), pp. 708–711. AVI Van Nostrand Reinhold Company, New York.

Wakebe, M. 1998. Feed for meat chickens and feed for laying hens. Japanese Patent Office, Application Heisei 8-179629. Jan. 27.

Walter, R., Schwartz, I.L., and Roy, L. 1973. Can selenoamino acids act as reversible biological antioxidants? *Ann. NY Acad. Sci.* **107**, 175–180.

Wang, Z.-J., Zhou, J., and An, P. 1992. Metabolic differences and similarities of selenium in blood and brain of the rat following the administration of different selenium compounds. *Biol. Trace Elem. Res.* **33**, 135–143.

Waschulewski, I.H. and Sunde, R.A. 1988. Effect of dietary methionine on tissue selenium and glutathione peroxidase on tissue selenium and glutathione peroxidase (EC 1.11.1.9) activity given selenomethionine. *Br. J. Nutr.* **60**, 57–68.

Whanger, P.D. 2002. Selenocompounds in plants and animals and their biological significance. *J. Am. Coll. Nutr.* **21**, 223–232.

Whanger, P.D. and Butler, J.A. 1994. Can physiological responses be used to assess the chemical forms of selenium consumed by humans, *In* "Proc. STDA 5th Internatl. Symp.", pp. 153–156, May 8–10. Brussels, STDA, Brussels.

Whanger, P.D., Green, J.L., and Butler, J.A. 1998. Should selenium enriched vegetables be consumed for the prevention of cancer. *In* "Proc. 6th Internat. Symp. Uses of Selenium and Tellurium" (Y. Palmieri, ed.), pp. 57–61, Scottsdale, AZ. Grimbergen.

WHO, 1996. *Report of a Joint FAO/IAEA/WHO Expert Consultation, Trace Elements in Human Nutrition and Health*, World Health Organization, Geneva, p. 343.

Wolffram, S.B., Berger, B., and Scharrer, E. 1989. Transport of selenoaminoacids and their sulfur analogues across the intestinal brush border membrane. *J. Nutr.* **119**, 706–712.

Yang, G. and Xia, Y. 1995. Studies on human dietary requirements and safe range of dietary intakes of selenium in China and their application in the prevention of related endemic diseases. *Biomed. Environ. Sci.* **8**, 187–201.

Yang, G. and Zhou, R. 1994. Further observations on the human maximum safe dietary selenium intake in a seleniferous region of China. *J. Trace Elem. Electrol. Health Dis.* **8**, 159–165.

Yang, G., Zhou, R., Yin, S., Gu, L., Liu, Y., and Liu, X. 1989a. Studies of safe maximal dietary Se intake in a seleniferous area in China. I. Selenium intake and tissue selenium levels of the inhabitants. *J. Trace Elem. Electrol. Health Dis.* **3**, 77–89.

Yang, G., Yin, S., Zhou, R., Gu, L., Liu, Y., and Liu, X. 1989b. Studies of safe maximal dietary Se intake in a seleniferous area in China. II. Relation between Se intake and the manifestation of clinical signs and certain biochemical alterations in blood and urine. *J. Trace Elem. Electrol. Health Dis.* **3**, 123–130.

Yang, X., Tian, Y., Ha, P., and Gu, L. 1997a. Determination of the selenomethionine content in grain and human blood. *Wei Sheng Yen Chiku and J. Hyg. Res.* **26**(2), 113–116.

Yang, X., Zhou, R., Ha, P., Tian, Y., Gu, L., and Xia, Y. 1997b. Method of cyanogen bromide-fluorimetric determination of trace amount of selenomethionine in grain and blood. *Weishung Yanjiu* **26**, 56–59.

Yin, S., Jin, Y., and Kenji, Y. 1996. Effect of dietary vitamin B6 on selenium bioavailability. IV. Effect of dietary vitamin B6 on selenium bioavailability from different chemical forms and lipid peroxidation in rats. *Weishung Yanjiu* **25**(5), 296–300.

Yu, S.Y., Chu, Y.J., and Li, W.G. 1988. Selenium chemoprevention of liver cancer in animals and possible human applications. *Biol. Trace Elem. Res.* **15**, 231–241.

Zayed, A.M., Lytle, C.M., and Terry, N. 1998. Accumulation and volatilzation of different chemical species of selenium by plants. *Planta* **206**, 284–294.

Zdansky, G. 1968. Studies in the synthesis of selenoamino acids. *Acta Univ. Upsalensis* **123**.

Zemanova, D., Simek, M., Illik, J., and Pavlata, L. 2000. Organic trace elements (Se) in rations for dairy cows. *In* "Mengen- und Spurenelemente, Arbeitstagung, 20th, Jena, Germany, Dec. 1–2, 2000" (M. Anke, ed.), pp. 636–644.

Zheng, O. and Wu, J. 1988. The determination of selenomethionine in selenium yeast by cyanogen bromide gas chromatography. *Biomed. Chromatogr.* **2**, 258–259.

Zheng, O., Wu, J., and Xie, L. 1989. A method for the indirect determination of trace bound selenomethionine in plants and some biological materials. *Anal. Biochem.* **178**, 77–81.