2
The Biology of Selenium

2.1 A Belated Recognition

The first clear indication that selenium plays a vital role in the metabolism of animals was obtained in the late 1950s when the element was shown by Schwartz and Foltz (1957) to be a key component of the so-called Factor 3, an active principle found in brewer's yeast able to replace vitamin E in preventing liver necrosis in rats and chickens. The discovery was a milestone in our understanding of the biological significance of selenium. Until then, it had been known only as a toxin, but was now shown to have a positive and presumably essential role in health. In quick succession, researchers were able to demonstrate that a variety of enzootic myopathies in cattle and sheep, as well as exudative diathesis in chickens (Patterson et al., 1957), that had been found to respond to vitamin E, could be controlled even more effectively by selenium (Andrews et al., 1968).

Another major step was taken in the early 1970s, when selenium was shown to be an essential component of glutathione peroxidase (GPX), an enzyme that provides antioxidant protection by reducing levels of hydroperoxides in cells (Rotruck et al., 1972). About the same time, it was reported that GPX was a tetramer containing about 4 g atoms of selenium per mole (Flohé et al., 1973). As well as demonstrating for the first time the central position of selenium in the structure of a functional protein, this finding appeared to offer an explanation for the "sparing" of vitamin E by selenium which also protected cells against oxidative damage (Combs, 2001). It seemed that now all that was needed to be known about the biological role of selenium had been discovered. How wrong this belief was has been shown by results of the vast amount of research on selenium that was to be carried out over the following 3 decades.

2.2 The Biological Role of Selenium in Prokaryotes

About the same time that mammalian GPX was demonstrated to be a selenoenzyme, selenium-dependent enzymes were also identified in certain microorganisms. One of these was the anaerobic bacterium *Clostridium sticklandii*, which

requires selenium for the synthesis of a key protein in its glycine reductase enzyme complex (Turner and Stadman, 1973). Of even more significance, in the light of subsequent findings, was the identification by Stadman's group of selenocysteine as the selenium moiety in the polypeptide of the glycine reductase. This was the first demonstration of the role of selenoamino acid as an essential residue in a selenoenzyme (Cone et al., 1976).

Selenoenzymes have subsequently been identified and isolated from other microorganisms. One of these, formate dehydrogenase in *Eschericia coli*, has been studied extensively, with results that have contributed considerably to our understanding of the biological role of selenium. Of particular significance was the discovery, again by Stadman's group, that genes for formate dehydrogenase contain an in-frame UGA codon that directs the cotranslational insertion of selenocysteine into protein (Zinoni et al., 1986).

2.3 Selenium in Plants

Certain lower plants, such as plankton algae, require selenium for growth (Lindström, 1948). One of these organisms, the obligate selenium-dependent dinoflagellate *Peridinium gatuense* has been used in ecological studies in Sweden as a bioassay of selenium bioavailability in freshwater (Lindström and Johansson, 1995). Selenium does not appear to be required for growth of *Saccharomyces cerevisiae*, although the yeast can take up large amounts from the medium in which it is cultured. The selenium is assimilated in competition with sulfur and is metabolized into a number of different organic compounds, the major product being selemomethionine (Demirici, 1999). Fungi, including those used as human food, can also accumulate and metabolize selenium, though apparently not requiring it for growth (Piepponen et al., 1983). Levels of about 10 µg/g (dry weight) are commonly recorded in mushrooms cultivated in normal compost, while those in compost enriched with sodium selenite more than 1,000 µg/g (dry weight) can be accumulated (László and Csába, 2004), Much of the selenium in mushrooms appears to be in the form of selenomethionine, with some in other organic forms (Werner and Beelman, 2001).

2.3.1 Selenium in Higher Plants

Selenium is apparently not required for growth by majority of higher plants, though there is still some doubt about this (Novoselov et al., 2002). It is, in fact, toxic to many plants, which cannot grow on seleniferous soils. However, a small number of species actually thrive on high-selenium soils and these do appear to require the element (Trelase and Trelase, 1939). These unusual plants are sometimes called *primary indicator plants* because their presence indicates that selenium is a component of the soil in potentially large amounts. Some of them are *hyperaccumulators* (Baker and Brookes, 1989), able to accumulate extraordinarily high levels of selenium, even, in some cases, from soil that contains relatively

little of the element (Brown and Shrift, 1982). Certain species of the vetch *Astragalus*, for example, have been found to contain more than 3,000 mg/kg (dry weight) in their leaves (Broyer et al., 1972). Ingestion of these so-called *locoweeds* by grazing animals can cause acute poisoning (Rosenfeld and Beath, 1961).

2.3.1.1 Selenium Accumulators and Nonaccumulators

Another group of plants can also grow on selenium-rich soils, but do not require it for growth. These can accumulate the element in their tissues, in some cases to relatively high levels. It is a somewhat ill-defined group, to which the names *secondary converter plants* and *facultative* or *secondary selenium absorbers* have been given. Among them are a number of grasses and other forage plants, such as alfalfa and clover, and plants used as human foods, such as garlic, onions, Swiss chard, broccoli, and other *brassicae*, as well as cereals, including barley, wheat, and rice. Since they are capable of accumulating potentially toxic levels of selenium, such plants can, in some seleniferous soil areas, pose a health problem to farm animals and humans (Ullrey, 1981).

Under normal field conditions, in the absence of high-selenium soil, forage and other crop plants will accumulate selenium, even though it is not required for their growth. Levels of uptake will depend on soil concentrations, as well as on certain other factors such as the chemical form of selenium present, as well as soil pH and moisture content. Crop plants grown on nonseleniferous soils normally contain between 0.01 and 1.0 mg/kg (dry weight) of selenium (Marschner, 1995). Even on soils with somewhat higher than normal levels of selenium, concentrations rarely exceed an upper limit of 1.0 mg/kg (Burau et al., 1988). In certain regions where selenium levels are low, such as in Finland and New Zealand, farmers add selenium to fertilizers used on their fields. This is not to promote plant growth, which the selenium will not do, but to increase levels in crops to meet the nutritional needs of consumers, both farm animals and humans (Oldfield, 1992).

2.3.2 Selenium Metabolism in Plants

In recent years, a considerable amount of research has been carried out on the uptake and metabolism of selenium by plants. The trigger for much of this work was the recognition, not only by scientists, but also by administrators, politicians, and, not least, the general public, especially in the USA, of the importance of selenium as an environmental contaminant. It followed the wide publicity given to the finding of extensive selenium pollution of water and plants, with disastrous consequences for birds and fish, in the wildlife refuge at Kesterton Reserve in California. The contamination resulted from high selenium levels in agricultural drainage water, runoff from neighboring farms that had been allowed to flow into the reservoir (Saiki and Lowe, 1987). There was considerable concern that vegetables and other crops produced in quantity on intensively irrigated farms in the San Joaquin Valley, one of the most productive agricultural areas in the country

(Oldfield, 1999), and other areas in central California might also be contaminated and be a health hazard for consumers. This concern triggered a significant escalation in interest among environmental scientists, encouraged by generous financial assistance from government and private sources. An informative review of the resulting research, especially as it relates to selenium metabolism in accumulator and nonaccumulator plants, by Terry and colleagues has been used to provide the basis of the following brief outline (Terry et al., 2000). The full paper, which cites some 175 original studies, is recommended to readers who wish to follow up aspects of the subject that are only briefly covered here.

2.3.2.1 Selenium Uptake and Transport in Plants

Selenate is taken up by plant roots from the soil by a process of active transport (Brown and Shrift, 1982). It competes with sulfur for uptake, both anions using a sulfate transporter in the root plasma membrane (Arvy, 1993). Organic forms of selenium, such as selenomethionine, are also taken up actively by plant roots. In contrast, transport of selenite does not appear to require the use of a sulfur transporter (Abrams et al., 1990).

Subsequent translocation of selenium within the plant is related to the form in which the element is supplied to the root. Selenate is more easily transported from the roots and much more is accumulated in the leaves than either selenite or organic selenium. Much of the selenite is retained in the roots where it is rapidly converted into organic forms, particularly selenomethionine (Zayed et al., 1998).

Distribution of selenium in various tissues differs between accumulator and nonaccumulator plants. In the former, the selenium is accumulated especially in young leaves, but later appears at higher levels in seeds than in other tissues, while, in nonaccumulators, such as cereals, levels in seeds and roots are usually the same (Beath, 1937).

2.3.2.2 Selenium Assimilation and Modification Within Plant Tissues

Although there is no strong evidence that selenium is an essential requirement for plant growth, it is nevertheless metabolized in a variety of ways once it is taken up into the plant tissues. These include, for example, nonspecific incorporation into selenoaminoacids and proteins, as well as synthesis of certain volatile selenium compounds, which can be released into the atmosphere from the plant's external surfaces (Zayed et al., 1999).

Selenium is translocated from the roots to the leaves via the xylem without undergoing any chemical transformation (De Souza et al., 1998). It is then reduced to selenide in the leaf chloroplasts, in a series of both enzymatic and nonenzymatic reactions, via glutathione (GSH) and the intermediate compound selenodiglutathione (GS-Se-SG). The selenide can then be converted into the selenoamino acid selenocysteine by coupling with *o*-acetylserine and then non specifically incorporated into protein (Ng and Anderson, 1979). Selenocysteine is also believed to be metabolized into selenomethionine, which likewise can be

incorporated into proteins, in place of methionine. The nonspecific replacement of cysteine and methionine in proteins by selenocysteine and selenomethione has been shown to occur readily in non accumulator plants treated with selenium (Eustice et al., 1981).

The volatile compound dimethylselenide (DMSe) is produced by methylation of selenomethionine in an enzymatic reaction that apparently occurs in the cytosol. Since roots volatilize selenium at a much faster rate than other tissues, DMSe precursors, which are synthesized in chloroplasts, must be transported downwards from the leaves for this to occur (Zayed and Terry, 1994).

While the initial steps in selenium uptake and conversion to selenocysteine are believed to be the same in both selenium accumulators and non accumulators, subsequent metabolic pathways differ. Unlike nonaccumulators, accumulators metabolize selenocysteine primarily into different nonprotein selenoamino acids (Brown and Shrift, 1982). Among these are selenomethylselenocysteine (Se-methylSeCys), selenocystathione, and the dipeptide, γ-glutamyl-seleno-methyl-selenocysteine (Terry et al., 2000).

2.3.2.3 Selenium Toxicity and Tolerance in Plants

Concentration of selenium in the tissues of plants at which they begin to show symptoms of toxicity, such as stunting, chlorosis, and withering of leaves, range from 2 mg/kg in nonaccumulators, such as rice, and 330 mg/kg in white clover (Mikkelson et al., 1989), to several thousand mg/kg in the accumulator *Astragalus bisulcatus* (Shrift, 1969).

In addition to selenium concentrations, other factors, such as the stage of growth, levels of sulphate in the soil, and the chemical form of selenium accumulated, determine the susceptibility of a particular plant to toxicity. Both selenite and selenate are the major forms that are toxic to nonaccumulators because they are readily absorbed and assimilated by the plants (Wu et al., 1998). The major mechanism of selenium toxicity is believed to be the incorporation of selenoamino acids, selenocysteine, and selenomethionine, into proteins in place of cysteine and methionine. Alterations in tertiary structure, resulting from differences in size and ionization properties between the sulfur and selenium atoms, probably have a negative effect on catalytic activity of certain important proteins (Brown and Shrift, 1982). Other ways in which selenium induces toxicity in plants are believed to be by interfering with chlorophyll synthesis (Padmaja et al., 1989) as well as with nitrate assimilation (Aslam et al., 1990). There is also evidence that selenium can interfere with production of glutathione, and thus reduce a plant's defense against hydroxyl radicals and oxidative stress (Bosma et al., 1991).

Tolerance by accumulators towards levels of selenium that would result in toxicity in nonaccumulators appears to be largely due to the reduction of intracellular concentrations of selenocysteine and selenomethionine, thus preventing their incorporation into proteins. This is brought about by converting the selenium into nonprotein selenoamino acids, such as selenocystathionine, or into the dipeptide

γ-glutamyl-seleno-methyl-selenocysteine (Nigam et al., 1969). There is some evidence that it may, to some extent, be achieved by compartmentation of the element in the form of selenate, or perhaps as nonprotein selenoamino acids, in vacuoles (Terry et al., 2000).

2.3.2.4 Volatilization of Selenium by Plants

Although it is not, strictly speaking, a tolerance mechanism, the ability of plants to convert selenium into volatile compounds that are then released into the atmosphere, thus reducing their selenium load, is an important metabolic activity of a variety of different plant types. The process have been intensively investigated in recent years, mainly in relation to its significance in what is known as phytoremedation of contaminated soil (Bañuelos et al., 1997). Rates of volatilization vary substantially between plant species and are related to a number of factors, including the concentration and chemical form of selenium and of sulfur in the soil, as well as to time of the year. In a laboratory study of the process in different crop species grown in solution culture, the highest rates of volatilization, between 300 and 350 μg Se/m^2 leaf area/day, were in rice, broccoli, and cabbage, while in beet, bean, lettuce, and onion they were >15 μg/m^2/day (Terry et al., 1992). High rates of volatilization have also been reported in the selenium accumulator *A. bisulcatus* (Duckart et al., 1992). In a field study of different plant species, the wetland *Salicornia bigelovii* (pickleweed) was found to have a rate of 420 μg Se/m^2 soil surface/day, 10 to 100 times greater than other plants, including cotton and Eucalyptus (Terry and Lin, 1999).

2.3.3 Selenium in Food Plants

While the mechanisms by which accumulator plants metabolize selenium in a variety of ways, and especially into volatile compounds, is of considerable interest to investigators dealing with the problem of environmental contamination, it is the ability of food plants, the major source of the element in most human diets, to take up this essential nutrient from the soil and store it in their tissues, that is of prime interest to nutritionists.

2.3.3.1 Crops Grown on Low-Selenium Soils

Where soil selenium levels are low, or the element occurs in a form that is not readily available for absorption by the roots, uptake by crops will be limited. In New Zealand, the selenium content of most of the arable land is low and, as a consequence, selenium levels in herbage are also low (Thomson and Robinson, 1980). Until steps were taken to improve soil levels by addition of sodium selenite to fertilizers, grazing on such lands resulted in selenium deficiency diseases in sheep and cattle. A similar situation was a major concern in Finland until it was also overcome by supplementation of fertilizers with selenium (Pykkö et al., 1988). Low-selenium agricultural soils have been reported in other countries also,

although usually on a lesser scale than in New Zealand and Finland, with similar effects on animal health. In Australia areas of selenium-deficient soils are found in many agricultural regions and require intervention, either by direct supplementation of animals or by addition of selenium to fertilizers (Langlands, 1987).

Low selenium levels in plant foods used directly for human consumption are implicated in serious health problems in areas of selenium-deficient soils in central and western China and neighboring regions. There, the two best-known selenium deficiency-related conditions in humans—Keshan and Kashin-Beck diseases—are endemic. Dietary intakes of selenium as low as 7 μg/day occur, 10 to 20 times less than intakes in many other countries in which selenium-responsive diseases in humans do not normally occur (Yang, 1991). The cause of the deficiency is reliance by inhabitants of such regions for up to 70% of their food intake on locally produced cereals. These often contain less than 0.02 μg Se/g, reflecting soil levels of about 0.1 μg/g. In contrast, in the USA, where soil levels in the major cereal growing regions are generally high, grains contain on average about 0.30 μg Se/g. This level of intake is more than enough to make a major contribution to meet the dietary requirements for selenium, even though cereals make up only about 30% of the normal American diet (Combs and Combs, 1986).

2.3.3.2 Crops Grown on Adequate-Selenium Soils

The selenium content of food plants grown on soils with an average level of the element in available form which is approximately 0.5 to 1.0 μg/g, according to what is known as the Wells rating scale (Wells, 1967), will generally be in a relatively narrow range of approximately 0.1 to 1.0 μg/kg. The range will vary somewhat between countries, depending on local soil conditions. In Australia, for instance, average selenium levels in wheat of 0.15 μg/g have been reported (Tinggi et al., 1992), compared to North American levels of 0.33 μg/g (Ferretti and Levander, 1974). In vegetables and fruits, Australian figures were 0.001 to 0.022 μg/g, somewhat lower than American findings of 0.004 to 0.063 μg/g in similar foods (Schubert et al., 1987).

2.3.3.3 Crops Grown on High-Selenium Soils

Cereals and other farm crops grown on selenium-rich soils may, under certain conditions, accumulate high levels of the element and even pose a threat of toxicity to consumers. Samples of cereals from seleniferous regions of South Dakota in the USA have been found to contain up to 30 μg Se/g (Byers, 1936). In seleniferous regions of Enshi County in China, rice containing 2.5 μg/g, maize flour 7.5 μg/g, and leafy vegetables up to 7.6 μg/g of selenium have been reported (Yang et al., 1989). However, even on seleniferous soil, not all crops will take up toxic levels of the element. The average selenium content of wheat plants sampled from an area of high-selenium soil in Montana, USA, was 1.9 μg/g, with a

maximum of 8 μg/g, even though there were a number of wild accumulator plants containing more than 1,000 μg Se/g (University of California Agricultural Issues Center, 1988). Even in the Chinese study of foods grown on high-selenium soil, several of the plants analyzed had less than 1 μg Se/g (Yang et al., 1989).

Rosenfeld and Beath (1961) in their comprehensive study of the distribution, properties, and health effects of selenium, described several cases of chronic selenium poisoning of people living in South Dakota. In all cases the source of the selenium was home-produced vegetables and other foods. Elimination of these foods from the diet led to recovery. The authors also referred to reports of chronic selenium poisoning in Columbia, South America, caused by consumption of locally produced foods grown in certain seleniferous regions. Soil selenium levels ranged from 12.6 to 20 μg/g and levels in crops were as much as 155 μg/g in wheat and 40 μg/g in barley. The problem, according to the authors, had actually been commented on as long ago as the 16th century when Fra Pedro Simon, a missionary priest, wrote, that "corn as well as other vegetables grow well and healthy but in some regions it is so poisonous that whoever eats it, man or animal, loses his hair. Indian women gave birth to monstrous-looking babies" (Simon, 1560). Rosenfeld and Beath noted that almost 400 years after Simon had made his observations, symptoms of selenosis, including hair and nail loss in humans and hoof damage in animals, continued to be recorded in Colombia: "reports in 1955 from one district described toxic corn and streams that had no animal life. Men and animals using the streams for drinking water showed loss of hair; small animals became sterile, and horses suffered hoof damage" (Rosenfeld and Beath, 1961).

2.3.3.4 The Risk of Selenosis in Humans From Eating Selenium-Rich Crops

The possibility that cereals and other crops grown on high-selenium soils and sold on the market without indication of their place of origin might poses a risk of selenosis to consumers first caused concern several decades ago. This was when information became available on the occurrence of selenium toxicity in farm animals and, to a lesser extent in humans, through consumption of selenium-enriched crops. That concern is still to be met today, as is evidenced by the publicity given to the problem of selenium contamination of farm produce grown in the San Joaquin Valley and other irrigated areas of central California (Bauer, 1997). It is also reflected in the inclusion of maximum permitted levels for selenium in the food standard regulations of many countries. However, extensive investigations have failed to find convincing evidence that selenium toxicity, resulting from consumption of naturally contaminated crops, is a real possibility for humans who consume a reasonably varied diet. As we shall see later, the occurrence of widescale, endemic human selenosis in Enshi County, a high-selenium region in Central China, has been attributed to consumption of a restricted diet of locally produced foods, of which selenium-rich cereals were a major part.

As has been noted by Oldfield (1990), though there are occasional occurrences of chronically toxic levels of selenium (above 5 μg/g) in cereals and other crops grown on seleniferous regions, in general, average figures on plant selenium con-

tents tend to be reassuring. In extensive surveys of many thousands of samples of North American wheat, levels of 1 μg/g, or less, were found, with a maximum of 1.5 μg/g. In global terms these are significant findings, since North American wheat, which is used extensively in many countries, is generally richer in selenium than the grains produced in most other parts of the world.

2.4 Selenium in Animal Tissues

The selenium content of animal tissues reflects that of the foods they consume. Animals raised on low-selenium feeds deposit relatively low concentrations of the element in their tissues and in their products, such as eggs and milk, while those on relatively high-selenium intakes yield products with higher concentrations. Organ meats usually accumulate more selenium than do other tissues, such as muscle (Combs and Combs, 1986). The principle chemical form of selenium in animal tissues is selenocysteine, unlike plant food in which selenomethionine predominates.

Once it has been ingested, the distribution of selenium within the body, of humans as well as of other animals, and also its absorption and excretion, depend on several factors, particularly the chemical form as well as on total amount of the element in the diet. In addition intake can be affected by the presence of certain other components of food, including sulfur, heavy metals, and vitamins (Underwood, 1977). Other factors, including sex, age, condition of health as well as nutritional status, can also affect the level of uptake and distribution in the body.

2.4.1 Absorption, Transport, and Excretion of Selenium

Absorption of selenium occurs mainly at the lower end of the small intestine. All forms of selenium, organic as well as inorganic, are readily absorbed. Overall absorption has been shown, in experimental animals and in humans, to be around 80%. There are differences, however, between levels of absorption, as well as of subsequent utilization, of the different chemical forms of the element. In general, organic compounds, such as selenomethionine, are absorbed more efficiently than are inorganic forms, particularly selenite, with uptake from the gastrointestinal tract of more than 90% of selenomethionine compared to about 60% of selenite (Stewart et al., 1987). Differences in chemical form also affect levels of retention in the body over time. It has been shown, in humans as well as in experimental animals, that selenomethionine is retained more efficiently than selenite or selenate, but is not as efficient in maintaining selenium status (Fairweather-Tait, 1997). Selenomethionine is also better retained in tissues, where it is incorporated into proteins, nonspecifically, in place of methionine, than is selenocysteine (Thomson, 1998).

There is some evidence of differences in the level of absorption of selenium if it is supplied along with food, rather than in isolation as organic or other supplements (Sirichakwal et al., 1985). It has also been reported that selenium is more

readily available if it is in plants rather than in animal foodstuffs (Young et al., 1982). Absorption can be affected by a number of dietary factors, in addition to the chemical form of the element. It is enhanced by the presence of protein, vitamin E, and vitamin A, and is decreased by sulfur, arsenic, mercury, guar gum, and vitamin C (Fairweather-Tait, 1997).

The major fate of all selenium absorbed from the diet, whatever its original chemical form or source, is to be incorporated into body proteins. The processes involved can be summarized briefly as follows: the ingested selenium is transported in the blood from the intestine to the liver. There it is reduced to selenide before being transported in the blood, bound to α- and γ-globulins to various organs and target tissues. It is then incorporated into specific selenoproteins, as selenocysteine, and, nonspecifically, as selenomethionine. The highest levels of selenium are deposited in red blood cells, liver, spleen, heart, nails, and tooth enamel. Excretion of absorbed selenium is mainly via the urine, with some loss in sweat, and also in hair. In addition, small amounts are lost through bilary, pancreatic, and intestinal secretions in feces (Linder, 1988).

2.4.2 Enteric Absorption of Selenium

There is uncertainty about the mechanisms of transport of dietary selenium across the intestinal epithelial membrane. Absorption of selenate appears to be by a sodium-mediated carrier transport mechanism shared with sulfur, while selenite uses passive diffusion (Fairweather-Tait, 1997). Both forms of inorganic selenium compete with inorganic sulfur compounds for absorption. In contrast, absorption of selenomethionine is active, using the same enzyme transport system as does methionine, with competition taking place between methionine and its seleno analog (McConnell and Cho, 1965).

Mechanisms of enteric absorption of selenoaminoacids other than selenomethionine are not clear. There is some evidence that the process is not active and is not physiologically controlled. It has been shown, in the case of the hamster, that selenocysteine transport across the duodenum wall will not proceed against a concentration gradient and that it is not inhibited by cysteine. Other findings point to an absence of any homeostatic or physiological control on enteric absorption of either organic or inorganic selenium. However, this view is not supported by reports that selenocysteine, like selenomethionine, may be actively transported in humans by the same transport mechanism as is used by its sulfur analog (Barbezat et al., 1984). It is possible that members of the SLC26 gene family of multifunctional anion exchangers, known to be involved in the transport of sulfate, as well as of other anions, also mediate transport of inorganic selenium in the intestinal brush border (Mount and Romero, 2004).

2.4.3 Transport of Selenium in the Body

Absorbed selenium is transported in the blood mainly bound to protein, following an initial reduction within the erythrocytes to selenide (Dreosti, 1986). The

process uses reduced glutathione and involves the enzyme glutathione reductase (Jenkins and Hidiroglou, 1972).

In humans, almost all the protein-bound selenium in blood is reported to be in the very low-density β-lipoprotein fraction, with smaller amounts bound to other proteins (Sandholm, 1974). However, distribution between these proteins appears to depend on the composition of the diet. Whanger et al. (1993) showed that nearly 50% of the selenium in plasma is associated with albumin in people who consume a diet in which selenomethionine is the main form of the element. There is also evidence that different proteins act as selenium carriers in other animal species (Young et al., 1982).

2.4.4 Selenium Distribution and Retention in the Human Body

The efficiency of retention of selenium in organs and other tissues appears to depend mainly on its chemical form and subsequent use. Thus selenomethionine is absorbed and retained more efficiently than are selenate and selenite. However, it is not as efficient at maintaining selenium status. The reason for this appears to be that though selenomethionine is retained in muscle and other tissue proteins to a greater extent than are the other forms, this retention is nonspecific, and the selenoamino acid is used immediately as a substitute for methionine in protein structure, not as a component of a functional protein (Thomson, 1998).

The total amount of selenium retained in an adult human body has been shown to range from about 2 to more than 20 mg. The total selenium content of a US adult, whose selenium intake can normally be expected to be high, has been calculated to be approximately 15 mg, with a range of 13 to 20.3 mg (Schroeder et al., 1970), while in New Zealand, where selenium intake is low, the range in women is 2.3 to 10 mg (Griffith et al., 1976).

There is evidence of an order of priority between organs for selenium uptake under different conditions of dietary supply. When intakes are adequate selenium concentrations in liver and kidney will be higher than in other organs. Overall, about 30% of tissue selenium is contained in the liver, 15% in kidney, 30% in muscle and 10% in plasma (WHO/FAO, 2002). At lower intakes, levels in liver and muscle can be markedly reduced, while remaining higher in kidney. It has been suggested that kidney has a ‘saturation level’ for selenium and a minimum requirement at the expense of other organs at low dietary intakes. This observation has been interpreted as indicating that the kidney plays a special role in selenium balance in the body (Oster et al., 1988).

It may be noted that although selenium concentrations are normally lower in muscle than in kidney and other organs, because of its relative bulk compared to other tissues, skeletal muscle appears to be a major storage compartment for selenium in the body (Whanger et al., 1993).

Using autopsy materials, Oster et al. (1988) determined selenium levels in organs of German adult male accident victims. They compared their results with data reported for other countries, as shown in Table 2.1. The results indicate that

TABLE 2.1 Selenium levels in body organs: International comparisons (μg/g, wet weight)

Country	Liver	Kidney	Muscle	Heart
Canada	–	0.390	0.840	0.370
USA	0.540	1.090	0.240	0.280
Japan	2.300	1.500	1.700	1.900
New Zealand	0.209	0.750	0.061	0.190
Germany	0.291	0.771	0.111	0.170

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

selenium levels in body organs, with the exception of kidneys, appear to be related to the country of residence and hence to dietary intakes. Since dietary selenium intakes are normally high in Japan, USA, and Canada, it can be expected that levels in liver, skeletal, and heart muscle will also be high. In contrast, in Germany, as in several other European countries, and New Zealand, intakes are low, with correspondingly low retention in body organs.

2.4.5 Selenium Levels in Blood

Animal experiments have shown that there is generally a positive correlation between blood selenium levels and dietary intakes (Linberg and Jacobsson, 1970). In humans, blood selenium can vary significantly between different populations, indicating a similar relationship with dietary intakes (Ihnat and Aaseth, 1989).

2.4.5.1 Selenium in Whole Blood

Table 2.2 presents a selection from the literature of data on whole blood selenium levels in subjects in different countries. They are all from persons in good health and exclude anyone suffering from overt selenium excess or deficiency. The reasons for these stipulations are that blood selenium levels are altered in certain disease states.

Selenium levels in whole blood have been shown to alter with change of residence from a high- to a low-selenium area. Thus, blood levels in visitors from the USA where selenium intake is high were found to drop in their initial year of residence in New Zealand where selenium intake is low from >0.15 to <0.10 μg/ml, a level found in permanent residents of the latter country (Rea et al., 1979).

2.4.5.2 Selenium in Serum and Plasma

Plasma and serum contain about 75% of the selenium of whole blood. Levels appear to be directly related to recent dietary intakes. They may also be age related and are altered in various diseases and health conditions (Lombeck et al., 1977). A world reference range, based on data published in the scientific literature for serum selenium levels of healthy adults, of 0.046 to 0.143 μg/ml has been

TABLE 2.2 Whole blood selenium levels (μg/ml) of healthy subjects living in different countries

Country	Reported levels (range or mean ± SD)	Reference
China	0.440–0.027	1
Finland (pre-1984)	0.081–0.056	2
New Zealand	0.072 ± 0.005	3
Sweden	>0.070	4
Australia	0.210–0.110	5
USA	0.300–0.150	6

References:

1. Yang, G., Wang, S., Zhou, R., and Sun, S., 1983, Endemic selenium intoxication of humans in China, *Am. J. Clin. Nutr.* **37**, 872–881.
2. Westermark, T., Rauni, P., Kirjarinta, M., and Lappalainen, L., 1977, Selenium content of whole blood and serum in adults and children of different ages from different parts of Finland, *Acta Pharmacol. Toxicol.* **40**, 465–475.
3. Thomson, C.D., Robinson. M.F., Butler, J.A., and Whanger, P.D., 1993, Long-term supplementation with selenate and selenomethionine: selenium and glutathione peroxidase (EC 1.11.1.9) in blood components of New Zealand women, *Br. J. Nutr.* **69**, 577–588.
4. Dickson, R.C. and Tomlinson, R.H., 1967, Selenium in blood and human tissue, *Clin. Chim. Acta* **16**, 311–7.
5. Judson, G.L., Thomas, W., and Mattschess, K.H., 1982, Blood selenium levels in Kangaroo Island residents, *Med. J. Aust.* **2**, 217.
6. Burk, R.F., 1984, Selenium, in: Black, G. (ed), *Nutrition Reviews: Present Knowledge in Nutrition,* 5th edn., Nutrition Foundation, Washington, DC, pp. 519–527.

proposed by the International Atomic Energy Agency (Iyengar and Woittiez, 1988). This is close to the range of 0.053 ± 0.0207 to 0.161 ± 0.019 μg/ml, derived from another critical survey of published results, which is believed to represent the true values for healthy adults in all but the most exceptional circumstances (Viesieck and Cornelis, 1989). Actual levels of selenium in serum/plasma recorded in residents of several different countries are shown in Table 2.3. The figures have been taken from the extensive data, based on clinical observations

TABLE 2.3 Plasma and serum selenium levels in different countries

Country	Selenium, μg/ml serum/plasma
Austria	0.067 ± 0.025
Australia	0.092 ± 0.015
Canada	0.135 ± 0.013
England	0.088 ± 0.021
France	0.083 ± 0.004
Italy	0.082 ± 0.023
New Zealand	0.065 ± 0.012
South Africa	0.177 ± 0.011
USA	0.140 ± 0.041
Zambia	0.040 ± 0.010

Adapted from Combs, G.F. Jr., 2001, Selenium in global food systems, *Br. J. Nutr.* **85**, 517–547.

reported from more than 70 countries worldwide, from Austria to Zambia, published by Combs (2001).

The apparent effects of disease on serum and plasma selenium levels are illustrated in Table 2.4. There is clearly not a direct relationship between state of health in various disease conditions and blood selenium levels. In some instances there is a reduction from normal levels, while in others the reverse occurs. Such changes are not necessarily a direct effect of a particular disease as such. They may be the result of, for instance, coexisting malnutrition or impaired metabolism. Moreover, it is not easy to interpret reports of clinical findings of changes in trace element levels in body fluids and tissues in conjunction with illnesses. There can be, moreover in some cases, at least a suspicion that not every clinical laboratory is sufficiently conscious of the fact that trace analysis is heavily fraught with technical peril (Versieck and Cornelis, 1989).

TABLE 2.4 Plasma and serum levels (μg/ml) in different disease states

Disease	Selenium level (control)	Reference
Cancer (gastrointestinal)	0.0486 ± 0.015 (0.0543 ± 0.016)	1
Diabetes (children)	0.074 ± 0.008 (0.065 ± 0.008)	2
Myocardial infarction	0.055 ± 0.015 (0.078 ± 0.011)	3
Chron's disease	0.110 ± 0.036 (0.096 ± 0.035)	4
Alcoholic liver cirrhosis	0.058 ± 0.011 (0.080 ± 0.011)	5
Renal disease	0.078 ± 0.016 (0.103 ± 0.018)	6

References:

1. Salonen, J.T., Alfthan, G., Huttunen, J.K., et al., 1984, Association between serum selenium and the risk of cancer, *Am. J. Epidemiol.* **129**, 342–354.
2. Gebre-Medhin, M., Ewald, U., Plantin, L., and Tumevo, T., 1984, Elevated serum selenium in diabetic children, *Acta Paediat. Scan.* **73**, 109–112.
3. Oster, O., Drexler, M., Schenk, J., et al., 1986, Serum selenium concentrations of patients with myocardial infarctions, *Ann. Clin. Res.* **18**, 36–40.
4. Deflandre, J., Weber, G., Delbrouck, J.M., et al., 1985, Trace elements in serum of patients with Chron's disease, *Gastroenterol. Clin. Biol.* **9**, 719–723.
5. Johanssons, U., Johanssons, F., Joelsson, B., et al., 1986, Selenium status in patients with liver cirrhosis and alcoholism, *Br. J. Nutr.* **55**, 227–231.
6. Sprenger, K.B.G., Krivan, V., Geiger, H., and Franz, H.E., 1985, Essential and non-essential trace elements in plasma and erythrocytes in patients with chronic uremic disease, *Nutr. Res.* Suppl. **1**, S-350.

2.4.5.3 Selenium Levels in Other Blood Fractions

Serum and plasma selenium levels are widely used clinically in the assessment of trace element status, although there is evidence that they do not necessarily reflect body stores or dietary intakes. Other blood fractions are also used in clinical investigations and have in some situations advantages over assessment of plasma and selenium (Bibow et al., 1993). Several investigators have reported the use of platelets for this purpose (Levander et al., 1983). Platelets have a relatively high concentration of selenium. They have a short life span and are believed to reflect recent changes in intake and body stores. They can be relatively easily separated from whole blood by gradient density centrifugation, a technique available in many clinical laboratories (Kaspareck et al., 1979).

2.4.6 Selenium Retention and Excretion from the Body

The biological half-life of selenium in the human body has been estimated to be approximately 100 days (Griffith et al., 1976). Actual retention times will depend on a number of factors, including present selenium status, the specific form in which the element is ingested, as well as the state of health of the subject. It has been shown in rats that the apparent whole-body retention of selenium is an average of several discrete processes, as each internal organ probably has its own rate of selenium turnover. For example, the half-life of ^{75}Se in rat kidney was found to be 38 days and 74 days in skeletal muscle, with a whole-body half-life of 55 days (Thomson and Stewart, 1973). It has been shown that the total body retention curve for selenium in humans can be resolved into a number of separate components (Yang et al., 1989).

Selenium is excreted from the body by three distinct routes, in urine via the kidneys, in feces from the gastrointestinal trace, and in expired air via the lungs. The amounts and proportions of each type of excretion depend on the level and form of the element in the diet (Beath et al., 1934).

2.4.6.1 Urinary Excretion of Selenium

The urinary pathway is the dominant excretion route for selenium in humans (Yang et al., 1989). The proportion of intake excreted in this way depends on the level of intake in the diet. When this is high, urinary excretion will also be high (Thomson and Robinson, 1986). At low levels of intake, half or less of the dietary selenium will appear in urine (Robinson et al., 1973). These findings point to the importance of renal regulation of selenium levels in the body. This view is supported by the fact that these levels are not, apparently, homeostatically controlled by the gut (Burk, 1976).

The renal system may have an important role in allowing the body to adapt to low dietary intakes of selenium by reducing excretion under such conditions. It has been shown that women with a low selenium status have low plasma clearance of the element and excrete it more sparingly than do women whose status is

high. There is evidence that New Zealanders appear to have adapted to their low-selenium environment by reducing urinary excretion, thus conserving their intake (Robinson et al., 1985). That such adaptation can develop surprisingly rapidly has been shown by results of depletion/repletion studies on healthy young men (Levander et al., 1981).

Several different chemical forms of selenium are found in urine, including selenomethionine, selenocysteine, selenite, selenate, and selenocholine (Robinson et al., 1985). Recently, selenium-containing carbohydrates (selenosugars) were detected in urine of rats fed selenite (Kobayashi et al., 2002). Many studies have reported trimethylselenonium (TMSe) to be the major form of selenium in urine and to account for up to 50% of the total normally present. Levels were believed to increase with higher intakes and it was generally thought to be produced as a way of excreting excess, potentially toxic selenium (Kuehnelt et al., 2005). The availability of improved analytical methods has produced results which changed this generally accepted view of selenium excretion. It is now believed that TMSe is not, under normal conditions, a significant constituent of human urine. However, it is produced in increasing quantities as selenium intake is increased and can be a biomarker of excessive intake (Suzuki et al., 2005). Three selenosugars have been identified in human urine and two of these, selenosugar **1** (methyl-2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside) and its deacylated analog selenosugar **3** (methyl-2-amino-2-deoxy-1-seleno-β-D-galactopyranoside), are believed to be major constituents. The third, selenosugar **2**, an analog of selenosugar **1**, appears to be a minor constituent. Although the significance of these findings is being investigated, and much more work is still to be done, the results so far obtained suggest that previously accepted pathways for human metabolism of selenium involving TMSe as the excretory end product may need to be reevaluated (Kuehnelt et al., 2005).

2.4.6.2 Fecal Excretion of Selenium

Fecal selenium consists largely of unabsorbed dietary selenium, along with selenium contained in bilary, pancreatic, and intestinal secretions (Levander and Baumann, 1966). It has been postulated that secretion of selenium in bile and its enterohepatic reabsorption may provide a mechanism, in addition to renal control, for conserving body stores. This could have major implications for populations with a low dietary intake (Dreosti, 1986).

Selenium excretion, whether by the urinary or fecal route, is affected by the chemical form of the element in the diet. Significant differences have been found in urinary excretion in rats fed different forms of selenium, as was shown in a series of trials carried out by Robinson's group. One week after feeding rats with selenite, selenocysteine, selenomethionine, "rabbit kidney" selenium, and "fish muscle" selenium, cumulative levels of selenium excreted in urine were 14, 14, 5, 7, and 6% of the absorbed dose, respectively (Richold et al., 1977). There is evidence that the proportion of inorganic to organic selenium that appears in urine is affected by the form of the element provided to the animal, at least when injec-

tion, rather than ingestion, is the entry route. When selenomethione was injected into rats, only 3% of the selenium detected in the urine was inorganic, compared to over 35% when selenate was injected (Nahapetian et al., 1983).

Selenium excretion in humans also appears to be affected by the form of the element ingested. Women volunteers who consumed 1 mg of selenium as selenate excreted 81% of the intake in urine, but less than a third of this when selenite was substituted for the selenate (Robinson et al., 1985). Similarly, over a 2-week period, volunteers fed microgram quantities of selenite excreted approximately twice as much total selenium, in urine and feces, as when fed equivalent amounts of selenomethionine (Yang et al., 1989).

2.4.6.3 Pulmonary Excretion of Selenium

Excretion of selenium via the pulmonary route in expired air and via the dermal route in sweat are of minor significance at normal levels of dietary intake. Excretion through the lungs occurs principally when intake is unusually high. Excess selenium is detoxified by successive methylation to form the volatile dimethyl selenide and other methylated species. The garlic-like odor of dimethyl selenide on the breath is characteristic of selenium intoxication (McConnel and Roth, 1966).

2.4.6.4 Losses of Selenium in Hair and Nails

Selenium is also lost to the body to a limited extent in hair and nails. These excretory pathways are of little consequence, from the point of view of homeostasis. However, they do have practical consequences. Selenium levels in nails and hair have been used in several studies as a measure of selenium status (Chen et al., 1980). They are considered to reflect long-term intake and provide a convenient, noninvasive assessment method (Longnecker et al., 1936). However, hair, in particular, must be used with caution, since selenium-containing hair treatments are widely used.

2.4.7 Selenium Pools and Stores in the Body

Although there is no evidence of a specific storage form of selenium, analogous to ferritin for iron, there are indications that the human body is capable of storing the element in different body pools. Women, for instance, who moved from the USA where selenium intake is high to New Zealand where selenium intake is low, experienced only a slow drop in blood selenium levels over a year before it reached the local level (Rea et al., 1979).

The storage seems to be due, at least in part, to the nonspecific incorporation of selenomethionine into the primary structure of body proteins and subsequently made available at a rate corresponding to muscle turnover and selenomethionine catabolism (Alfthan et al., 1991). The storage appears to occur in muscle, kidney, and erythrocytes (Whanger et al., 1993).

References

Abrams, M.M., Shennan, C., Zazoski, J., and Burau, R.G., 1990, Selenomethionine uptake by wheat seedlings, *Agron. J.* **82**, 1127–1130.

Andrews, E.D., Hartley, W.J., and Grant, A.B., 1968, Selenium-responsive diseases in animals in New Zealand, *NZ J. Vet. Med.* **16**, 3–17.

Alfthan, G., Aro, A., Arvillomi, H., and Huttunen, J.K., 1991, Selenium metabolism and platelet glutathione peroxidase activity in healthy Finnish men: effects of selenium yeast, selenite and selenate, *Am. J. Clin. Nutr.* **53**, 120–125.

Arvy, M.P., 1993, Selenate and selenite uptake and translocation in bean plants (*Phaseolus vulgaris*), *J. Exp Bot.* **44**, 1083–1087.

Aslam, M., Harbit, K.B., and Huffaker, R.C., 1990, Comparative effects of selenite and selenate on nitrate assimilation in barley seedlings, *Plant Cell. Environ.* **13**, 773–782.

Baker, A.J.M. and Brookes, R.R., 1989, Terrestrial higher plants which accumulate metallic elements – a review of their distribution, ecology and phytochemistry, *Biorecovery* **1**, 81–126.

Bañuelos, G.S., Ajwa, H.A., Mackey, M., et al., 1997, Evaluation of different plant species used for phytoremediation of high soil selenium, *J. Environ. Quality* **26**, 639–646.

Barbezat, G.B., Casey, C.E., Reasbeck, P.G., et al., 1984, Selenium, in: Solomons, N.W. and Rosenberg, I.H. (eds), *Absorption and Malabsorption of Mineral Nutrients*, Liss, New York, pp. 213–258.

Bauer, F., 1997, Selenium and soils in the western United States, *Electronic Green J.* **7**, 1–5, http://egj.lib.uidaho.edu/egj07/bauer.htm

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

Beath, O.A., Draise, J.H., Eppson, F., et al., 1934, Certain poisonous plants of Wyoming activated by selenium and their association with respect to soil types, *J. Am. Pharmacol. Assoc.* **23**, 94–97.

Bibow, K., Meltzer, H.M., Mundal, H.H., et al., 1993, Platelet selenium as indicator of wheat selenium intake, *J. Trace Elem. Electrolytes Health Dis.* **7**, 171–176.

Bosma, W., Svchupp, R., De Kok, L.J., and Rennenberg, H., 1991, Effect of selenate on assimilatory sulfate reduction and thiol content in spruce needles, *Plant Physiol. Biochem.* **29**, 131–138.

Brown, T.A. and Shrift, A., 1982, Selenium: toxicity and tolerance in higher plants, *Biol. Revs.* **57**, 59–84.

Broyer, T.C., Johnson, C.M., and Hudson, R.P., 1972, Selenium and nutrition of *Astragalus* I: effects of selenite or selenate supply on growth and selenium content, *Plant Soil* **36**, 635–649.

Burau, R.G., McDonald, A., Jacobson, A., et al., 1988, Selenium in tissues of crops sampled from the west side of the San Joaquin Valley, California, in: Tanji, K.K., Valoppi, L., and Woodring, R.C. (eds), *Selenium Contents in Animal and Human Food Crops Grown in California*, University of California Division of Agriculture and Natural Resources, Berkeley, CA, pp. 61–67.

Burk, R.F., 1976, Selenium in man, in: Prasad, A.S. (ed.), *Trace Elements in Human Health and Disease*, Academic Press, New York, pp. 105–133.

Byers, H.G., 1936, Selenium occurrence in certain soils in the United States, with a discussion of certain topics, *US Department of Agriculture Technical Bulletin*, No. 530, USDA, Washington, DC, pp. 1–8.

Chen, X., Yang, G.Q., Chen, J., et al., 1980, Studies on the relation of selenium and Keshan disease, *Biol. Trace Elem. Res.* **2**, 91–107.

Combs, G.F., 2001, Selenium in global food systems, *Br. J. Nutr.* **85**, 517–547.

Combs, G.F. Jr. and Combs, S.B., 1886a, Selenium in foods and feeds, in: Combs, G.F., Jr. and Combs, S.B. (eds), *The Role of Selenium in Nutrition*, Academic Press, New York, pp. 41–54.

Combs, G. and Combs, S., 1986b, *The Role of Selenium in Nutrition*, Academic Press, New York.

Cone, J.E., Martin del Rio, R., and Stadman, T.C., 1976, Selenocysteine in glycine reductase, *Proc. Natl Acad. Sci. USA* **73**, 2659–2663.

Demirici, A., 1999, Enhanced organically bound selenium yeast production by feed-batch fermentation, *J. Agric.Food Chem.* **47**, 2496–2500.

De Souza, M.P., Pilon-Smits, E.A.H., Lytle, C.M., et al., 1998, Rate limiting steps in selenium assimilation and volatilization by Indian mustard, *Plant Physiol.* **117**, 1487–1494

Dreosti, I, 1986, Selenium, *J. Food Nutr.* **43**, 60–78.

Duckart, E.C., Waldron, L.J., and Donner, H.E., 1992, Selenium uptake and volatilization from plants growing in soil, *Soil Sci.* **153**, 94–99.

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

Fairweather-Tait, S., 1997, Bioavailability of selenium, *Eur. J. Clin. Nutr.* **51**, S20–S23.

Ferretti, R.J. and Levander, O.A., 1974, Effect of milling and processing on the selenium content of grains and cereal products, *J. Agric. Food Chem.* **22**, 1049–1051.

Flohé, L., Gunzler, W.A., and Schock, H., 1973, Glutathione peroxidase: a selenoenzyme, *FEBS Lett.* **32**, 132–134.

Griffith, N.M., Stewart, R.D.H., and Robinson, M.F., 1976, The metabolism of ^{75}Se-selenomethionine in four women, *Br. J. Nutr*. **35**, 373–382.

Ihnat, M. and Aaseth, J., 1989, in: Ihnat, M. (ed.), *Occurrence and Distribution of Selenium*, CRC Press, Boca Raton, FL, pp. 169–212.

Iyengar, V. and Woittiez, J., 1988, Trace elements in human clinical specimens: evaluation of literature data to identify reference values, *Clin. Chem.* **34**, 474–481.

Jenkins, K.J. and Hidiroglou, M., 1972, Comparative metabolism of ^{75}Se-selenite, ^{75}Se-selenate and ^{75}Se-selenomethionine in bovine erythrocytes, *Can. J. Physiol. Pharmacol.* **50**, 927–935.

Kaspareck, K., Iyengar, G.V., Keiem, J., et al., 1979, Elemental composition of platelets. Part iii. Determination of Ag, Cd, Co, Cr, Mo, Rb, Sb and Se in normal human platelets by neutron activation analysis, *Clin. Chem.* **25**, 711–715.

Kobayashi, Y., Ogra, Y., Ishiwata, K., et al., 2002, Selenosugars are key and urinary metabolites for Se excretion within the required to low-toxic range, *Proc. Natl Acad. Sci. USA* **99**, 15932–15936.

Kuehnelt, D., Kienzl, N., Traar, P., et al., 2005, Selenium metabolites in human urine after ingestion of selenite, L-selenomethionine, or DL-selenomethionine: a quantitative case study by HPLC/ICPMS, *Anal. Bioanal. Chem.* **383**, 235–246.

Langlands, J.P., 1987, Recent advances in copper and selenium supplements in grazing ruminants, in: Farrell, D. (ed.), *Proceedings of Recent Advances in Animal Nutrition Conference*, University New England, Armidale, New South Wales, Australia, May, pp. 1–8.

László, R. and Csába, H., 2004, Iodine and selenium intake from soil to cultivated mushrooms, *2nd Intnational Symposium – Trace Elements in Food, Brussels, Belgium, 7–8 October, Abstracts*, European Commission Joint Research Centre, Brussels, Belgium, p. 21.

Levander, O.A., Alfthan, G., Arvilommi, H., et al., 1983, Bioavailability of selenium in Finnish men as assessed by platelet glutathione peroxidase and other blood parameters, *Am. J. Clin. Nutr.* **37**, 887–897.

Levander, O.A. and Baumann, C.A., 1966, Selenium metabolism VI. Effect of arsenic on excretion of selenium in the bile, *Toxicol. Appl. Pharmacol.* **9**, 106–115.

Levander, O.A., Sutherland, B., Morris, V.C. and King, J.C., 1981, Selenium balance in young men during selenium depletion and repletion, *Am. J. Clin. Nutr.* **34**, 2662–2669.

Linberg, P. and Jacobsson, S.O., 1970, Relationship between selenium content of forage, blood and organs of sheep, and lamb mortality rates, *Acta Vet. Scandinavica* **11**, 49–58.

Linder, M.C., 1988, *Nutritional Biochemistry and Metabolism*, Elsevier, New York, p. 177.

Lindström, K., 1948, Selenium as a growth factor for plankton in laboratory experiments and in some Swedish lakes, *Hydrobiology* **101**, 35–38.

Lindström, K. and Johansson, E., 1995, Improved techniques for analysis of selenium in fresh waters and biological materials, in: *Proc. STDA's Intnl. Symp, 8–10 May*, Brussels, Selenium-Tellurium Development Association, Grimbergen, Belgium, pp. 281–286.

Lombeck, I., Kasparek, K., Harbisch, H.D., et al., 1977, The selenium status of healthy children. I. Serum concentrations at different ages: activity of glutathione peroxidase of erythrocytes at different ages: selenium content of food of infants, *Eur. J. Pediat.* **125**, 81–89.

Longnecker, M.P., Stram, D.O., and Taylor, P.R., 1996, Use of selenium concentrations in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake, *Epidemiology* **7**, 384–390.

McConnell, K.P. and Cho, G.J., 1965, Transmucosal movement of selenium, *Am. J. Physiol.* **208**, 1191–1195.

McConnell, K.P. and Roth, D.M., 1966, Respiratory excretion of selenium, *Proc. Soc. Exp. Biol. Med.* **123**, 919–921.

Marschner, H., 1995, *Mineral Nutrition of Higher Plants*, Academic Press, London, pp. 430–433.

Mikkelson, R.L., Page, A.l., and Bingham, A.T., 1989, Factors affecting selenium accumulation by agricultural crops, *Soil Sci. soc. Am. Special Publications* **23**, 65–94.

Mount, D.B. and Romero, M.F., 2004, The SLC26 gene family of multifunctional anion exchangers, *Pflugers Archives* **447**, 710–721.

Nahapetian, A.T., Janghorbani, M., and Young, V.R., 1983, Urinary trimethylselenonium excretion by the rat: effect of level and sources of ^{75}Se-selenium, *J. Nutr.* **113**, 401–411.

Ng, B.H. and Anderson, J.W., 1979, Light-dependent incorporation of selenite and sulphite into selenocysteine and cysteine by isolated pea chloroplasts, *Phytochemistry* **17**, 2069–2074.

Nigam,S.N., Tu, J-I., and McConnell, W.B., 1969, Distribution of selenomethylcysteine and some other amino acids in species of *Astragalus*, with special reference to their distribution during the growth of *A. bisulcatus*, *Phytochemistry* **8**, 1161–1165.

Novoselov, S.V., Rao, M., Onoshoko, N.V., et al., 2002, Selenoproteins and selenocysteine insertion in the model plant system, *Chlamydomonas reinhardii, EMBO J.* **21**, 3681–3693.

Oldfield, J.E., 1999, *Selenium in agriculture: the early years. A.L. Moxton Honorary Lecture*, Ohio State Uni. Extension Research, http://ohioline.osu/sc167/sc167-04.html

Oldfield, J.E., 1992, *Selenium in Fertilizers,* Selenium–Tellurium Development Association, Grimbergen, Belgium.

Oldfield, J.E., 1990, *Selenium: its Uses in Agriculture, Nutrition and Health and the Environment*, Selenium–Tellurium Development Association, Grimbergen, Belgium.

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

Padmaja, K., Prasad, D.D.K., and Prasad, A.R.K., 1989, Effect of selenium on chlorophyll biosynthesis in mung bean seedlings, *Phytochemistry* **28**, 3321–3324.

Patterson, E.L., Milstrey, R., and Stokstad, E.L., 1957, Effect of selenium in preventing exudative diathesis in chicks, *Proc. Soc. Exp Biol. Med.* **95**, 617–620.

Piepponen, S., Liukkonen-Lilja, H., and Kunsi, T., 1983, The selenium content of edible mushrooms in Finland, *Zeitschrift für Lebensmitteluntersuchung und-Forschung* **177**, 257–260.

Pykkö, K., Tuimala, R., Kroneld, R., et al., 1988, Effect of selenium supplementation to fertilizers on the selenium status of the population in different parts of Finland, *Eur. J. Clin. Nutr.* **42**, 571–579.

Rea, H.M., Thomson, C.D., Campbell, D.R., and Robinson, M.F., 1979, Relationship between erythrocyte selenium concentrations and glutathione peroxidase (EC 1.11.1.9) activities of New Zealand residents and visitors to New Zealand, *Br. J. Nutr.* **42**, 201–208.

Richold, M., Robinson, M. F., and Stewart, R.D.H., 1977, Metabolic studies in rats of ^{75}Se incorporated in vivo into fish muscle, *Br. J. Nutr.* **388**, 19–29.

Robinson, M.F., McKenzie, J.M., Thomson, C.D., and van Rij, A.L., 1973, Metabolic balance of zinc, copper, cadmium, iron, molybdenum and selenium in young New Zealand women, *Br. J. Nutr.* **30**, 195–205.

Robinson, J.R., Robinson, M.F., Levander, O.A., and Thomson, C.D., 1985, Urinary excretion of selenium by New Zealand and North American subjects on differing intakes, *Am. J. Clin. Nutr.* **41**, 1023–1031.

Rosenfeld, I. and Beath, O.A., 1961, *Selenium, Geobotany, Biochemistry, Toxicity, and Nutrition*, Academic Press, New York, pp. 7–8.

Rotruck, J.T., Ganther, H.E., Swanson, A.B., et al., 1972, Selenium: biochemical role as a component of glutathione peroxidase, *Science* **179**, 588–590.

Saiki, M.K. and Lowe, T.P., 1987, Selenium in aquatic organisms from subsurface agricultural drainage water, San Joaquin Valley, California, *Arch. Environ. Contam. Toxicol.* **19**, 496–499.

Sandholm, M., 1974, Selenium carrier proteins in mouse plasma, *Acta Pharmacol. Toxicol.* **35**, 427–431.

Schroeder, H.A., Frost, D.V., and Balassa, J.J., 1970, Essential trace elements in man: selenium, J. *Chronic Dis.* **23**, 227–243.

Schubert, A., Holden, J.M., and Wolf, W.R., 1987, Selenium content of a core group of foods based on a critical evaluation of published analytical data, *J. Am. Diet. Assoc.* **87**, 285–299.

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

Shrift, A., 1969, Aspects of selenium metabolism in higher plants, *Ann. Rev. Plant Physiol.* **20**, 475–494.

Simon, P.F., 1560, Noticias historiales de las conquistas de tierre en las Indias occidentales, *Biblioteca Autores Colombianos*, Vol. 4, Kelly Publishing, Bogota, Colombia, 1953, pp. 226–254, cited in: Rosenfeld, I. and Beath, O.A., 1961, *Selenium, Geobotany, Biochemistry, Toxicity, and Nutrition*, Academic Press, New York.

Sirichakwal, P.P., Young V.P., and Janghorbani, M., 1985, Absorption and retention of selenium from intrinsically labelled egg and selenite as determined by stable isotope studies in humans, *Am. J. Clin. Nutr.* **41**, 264–269.

Stewart, R.D.H., Griffiths, N.M., Thomson, C.D., and Robinson, M.F., 1987, Quantitative selenium metabolism in normal New Zealand women, *Br. J. Nutr.* **40**, 45–54.

Suzuki, K.T., Kurasaki, K., Okazaki, N., and Ogra, Y., 2005, Selenosugar and trimethylselenonium among urinary metabolites: dose- and age-related changes, *Toxicol. Appl. Pharmacol.* **206**, 1–8.

Suzuki, K.T. and Ogra, Y., 2002, Metabolic pathways for selenium in the body: speciation by HPLC-ICP MS with enriched Se, *Food Add. Contam.* **19**, 974–983.

Terry, N. and Lin, Z.Q., 1999, *Managing High Selenium in Agricultural Drainage Water by Agroforestry Systems: Role of Selenium Volatilization*, Report of the Californian State Department of Water Resources, Sacramento, California, cited in: Terry, N., Zayed, A.M., De Souza, M.P., and Tarun, A.S., 2000, Selenium in higher plants, *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **51**, 401–432.

Terry, N., Carlson, C., Raab, T.K., and Zayed, A.M., 1992, Selenium uptake and volatilization among crop species, *J. Environ. Quality* **21**, 341–344.

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

Thomson, C.D., 1998, Selenium speciation in human body fluids, *Analyst* **123**, 827–831.

Thomson, C.D. and Robinson, M.F., 1986, Urinary and faecal excretions and absorptions of a large supplement of selenium: superiority of selenate over selenite, *Am. J. Clin. Nutr.* **44**, 659–663.

Thomson, C.D. and Robinson, M.F., 1980, Selenium in human health and disease with emphasis on those aspects peculiar to New Zealand, *Am. J. Clin. Nutr.* **33**, 303–323.

Thomson, C.D. and Stewart, R.D.H., 1973, Metabolic studies of ^{75}Se-selenomethionine and ^{75}Se-selenite in rat, *Br. J. Nutr.* **30**, 139–147.

Tinggi, U., 2005, Selenium toxicity and its adverse health effects, in: Preedy, R. and Watson, R.R. (eds), *Reviews in Food and Nutrition Toxicity*, Taylor & Francis, Boca Raton, FL, pp. 29–55.

Tinggi, U., Reilly, C., and Patterson, C.M., 1992, Determination of selenium in foodstuffs, using spectrofluorimetry and hydride generation atomic absorption spectrometry, *J. Food Comp. Anal.* **5**, 269–280.

Trelase, S.F. and Trelase.H.M., 1939, Physiological differentiation in *Astragalus* with reference to selenium, *Am. J. Bot.* **26**, 530–535.

Turner, D.C. and Stadman, T.C., 1973, Selenium a requirement for glycine reductase activity in *Clostridium sticklandii*, *Arch. Biochem. Biophys.* **154**, 366–381.

Ullrey, D.E., 1981, Selenium in the soil-plant-food chain, in: Spallholz, J.E., Martin, J.L., and Ganther, H.E. (eds), *Selenium in Biology and Medicine*, Avi Publishing, Westport, VA, pp. 176–191.

Underwood, E.J., 1977, *Trace Elements in Human and Animal Nutrition*, 4th edn., Academic Press, London, pp. 302–46.

University of California Agricultural Issues Center, 1988, Selenium, human health and agricultural issues, in: *Resources at Risk in the San Joaquin Valley*, Uni. California, Davis, CA, pp. 1–23.

Versieck, J. and Cornelis, R., 1989, *Trace Elements in Human Plasma or Serum*, CRC Press, Boca Raton, FL, pp. 2–3.

Wells, N., 1967, Selenium in horizons of soil profiles, *NZ J. Sci.* **10**, 142–153.

Werner, A.R. and Beelman, R.B., 2001, Growing selenium-enriched mushrooms as ingredients for functional foods or dietary supplements, *Int. J. Med. Mushrooms* **3**, 112–120.

Whanger, P., Xia, V., and Thomson, C., 1993, Metabolism of different forms of selenium in humans, *J. Trace Elem. Electrolytes Health Dis.* **7**, 121–125.

WHO/FAO, 2002, *Human Vitamin and Mineral Requirements: Report of a Joint WHO/FAO Expert Committee*, World Health Organization/Food and Agricultural Organisation, Rome, pp. 235–255.

Wu, L., Huang, Z.Z., and Burau, R.G., 1988, Selenium accumulation and selenium-salt co-tolerance in five grass species, *Crop Sci.* **28**, 517–522.

Yang, G.Q., 1991, Diet and naturally-occurring human diseases caused by inadequate intake of essential trace elements, in: *Proceedings of the 6th Asian Congress of Nutrition*, Kuala Lumpur, Malaysia, 16–19 Sept, pp. 79–92.

Yang, G., Wang, S., Zhou, R., and Sun, S., 1983, Endemic selenium intoxication of humans in China, *Am. J. Clin. Nutr.* **37**, 872–881.

Yang, G., Zhou, R. Gu, L., et al., 1989, Studies of safe maximal daily dietary selenium intake in a seleniferous area of China. 1. Selenium intake and tissue selenium levels of the inhabitants, *Trace Elem. Electrolytes Health Dis.* **37**, 77–87.

Young, V.S., Nahapetian, A., and Janghorbani, M., 1982, Selenium bioavailability with reference to human nutrition, *Am. J. Clin. Nutr.* **35**, 1076–1088.

Zayed, A.M. and Terry, N., 1994, Selenium volatilization in roots and shoots: effects of shoot removal and sulfate level, *J. Plant Physiol.* **143**, 8–14.

Zayed, A., Lytle, C.M., and Terry, N., 1998, Accumulation and volatilization of different chemical species of selenium by plants, *Planta* **206**, 284–292.

Zayed, A.M., Piton-Smits, E.A.H., De Souza, M.P., et al., 1999, Remediation of selenium-polluted soils and waters by phytovolatilization, in: Terry, N. and Bañuelos, G. (eds), *Phytoremediation of Metal-Contaminated Water and Soils*, CRC Press, Boca Raton, FL, pp. 61–83.

Zinoni, F., Birkmann, K., Stadman, T.C., and Bock, A., 1986, Cotranslational insertion of selenocysteine into formate dehydrogenase from *Eschericia coli* directed by a UGA codon, *Proc. Natl Acad. Sci.* **84**, 4650–4654.