Selenium consumed by humans in foods and in supplements exists in a number of different organic and inorganic forms including selenomethionine, selenocysteine, selenate and selenite. Animal and human studies have established that the bioavailability of the selenium depends upon the chemical form, which also influences the distribution of selenium in the body. These studies have included urinary excretion of selenium following ingestion of different forms of selenium, and the response of tissue selenium concentrations and activities of functional selenoproteins to these selenium compounds. Selenomethionine is retained in tissue proteins to a greater extent than selenocysteine and the inorganic forms, but the retention is not necessarily immediately available for functional selenoproteins. A number of other factors besides chemical form may also influence the bioavailability and distribution of selenium, including other dietary components, selenium status, physiological status and species. Knowledge of these factors and of speciation of selenium in foods, tissues and functional selenoproteins is important for the accurate assessment of selenium status. Speciation of selenium also has implications with respect to the determination of selenium requirements and to the investigation of relationships between selenium status and health and disease.

Keywords: Speciation; human; selenium; selenoproteins; selenium status; selenium requirements

It has now been well established that the bioavailability of selenium and its distribution in the organism depend to a large extent on its chemical form in the diet. The chemical species will therefore have an impact on its physiological behaviour. The importance of speciation for selenium encompasses the form of the trace element in food and in supplements, absorption, metabolism in the body and bioavailability for functional selenoproteins. The toxicity of selenium is also dependent on the chemical form in which it is ingested. This paper reviews, from a historical perspective, what is currently known about the speciation of selenium in human body fluids and implications for the assessment of nutritional status, the determination of selenium requirements and investigations of the relationships between selenium and health and disease.

Speciation of selenium and urinary excretion

Our first introduction to the importance of speciation of selenium compounds came in early studies in New Zealand of urinary excretion of selenium following varying sized doses of different forms of selenium, selenomethionine, selenite and selenate, which showed that there were major differences, not just in the absorption of these forms but also in the way in which they were handled in the body, ultimately resulting in urinary excretion. The absorption of dietary selenium in the form of the selenoamino acid selenomethionine in solution was > 95%. The apparent absorption of selenate-selenium was also high at > 90%, but the absorption of selenite-selenium was only about 60% of the total dose. Selenomethionine is absorbed by an active transport system shared with methionine, whereas absorption of inorganic selenium is by a passive process. The reason for the difference in absorption of selenite and selenate was not clear at the time. However, in a later study a large dose of ascorbic acid was shown to reduce the availability of selenite almost to zero, indicating that the absorption of this form may be more susceptible to other dietary factors. The inorganic forms of selenium were absorbed rapidly but were equally rapidly excreted in urine, in contrast to selenomethionine which was retained in the body. The total recovery in the urine and faeces of selenate and selenite was 82–95% of the total dose, whereas only 26% of the selenomethionine was recovered. The reasons for this were to be found later.

We extended these studies using minute doses of radioactively labelled selenium and later enriched stable isotopes which gave us insight into the longer term kinetics of selenium in the body. These studies confirmed our previous observations that 75Se from selenomethionine was more completely absorbed and had a greater retention and smaller endogenous urinary and faecal losses than 75Se from selenite, and these differences persisted throughout the study of over 20 weeks. These findings differed from those obtained in rats in which, after an initial period, 75Se from selenite was metabolised similarly to that from selenomethionine.

Later, when trimethylselenonium ion was identified as a urinary metabolite of selenium, we measured this in the urine of supplemented individuals. However, it was soon realised that this metabolite was of significance only at high intakes of selenium and thus was a marker of toxic rather than normal dietary intakes of selenium. Other species found in urine are selenomethionine, selenite, selenate, selenoamino acids and selenocholine, but the significance of these has not been studied. Knowledge of the different selenium species in urine can give information about the biochemical conversion of the element and detoxification mechanisms involved.

Urinary excretion can also be used to assess selenium status and we can estimate the total dietary intake as twice the daily urinary excretion. However, the extent to which this relationship is influenced by the dietary form at normal dietary intakes is less clear, partly because of the lack of knowledge of chemical forms of selenium in food.

Speciation of selenium and blood proteins

At the same time we were investigating the effects of all these dietary forms of selenium on blood selenium concentrations and the activity of the then newly discovered selenoenzyme glutathione peroxidase. It soon became obvious that not only were absorption and urinary excretion influenced by the chemical form of ingested selenium, but also the distribution of selenium in tissue proteins in the body.

We had earlier shown a close correlation between blood selenium levels and activities of glutathione peroxidase in New Zealand...
Zealand residents, at least up to blood selenium concentrations of about 100 ng ml⁻¹.¹³ However, as the selenium concentrations increased the glutathione peroxidase activities did not increase further.¹⁴ When we compared the effects of long-term supplementation of selenate and selenomethionine on blood selenium levels and glutathione peroxidase activities in New Zealand women, we found that selenomethionine increased blood selenium levels to a much greater extent than did selenite, but that glutathione peroxidase activities plateaued at similar levels for both dietary forms in erythrocytes and plasma.¹⁵ In other words, the bioavailability of the two forms with respect to glutathione peroxidase activity was similar.

Chromatography of erythrocyte lysates from these subjects indicated that the majority of the selenium was with haemoglobin in women taking selenomethionine but was equally distributed between glutathione peroxidase and haemoglobin in women taking selenate.¹⁶ Thus the percentage of selenium associated with glutathione peroxidase was found to be greater in erythrocytes of women taking selenate than in those of women taking selenomethionine. A similar pattern was seen in plasma. Gel filtration of plasma from women taking selenomethionine revealed two major selenium-containing peaks with most of the selenium in the second protein peak, which was identified as albumin.¹⁶ In contrast, the first peak contained most of the selenium in plasma from women taking selenate. This was identified as selenoprotein P. A third, smaller, peak represented glutathione peroxidase; there was no difference in selenium accumulation in plasma glutathione peroxidase between the two groups, but the percentage of selenium in this glutathione peroxidase peak was greater for selenate than for selenomethionine supplements.

We had earlier shown in another study comparing the distribution of selenium between erythrocyte fractions of people living in Oregon with those in New Zealand¹⁷ that when lysates were subjected to gel filtration, no differences in the selenium content of the glutathione peroxidase fraction were detected between Oregon and New Zealand samples. However, the selenium content was significantly higher in the haemoglobin fraction of the Oregon samples, suggesting that the greater amount of selenium in the whole blood was due to the higher content in the haemoglobin fraction. The calculated percentage of selenium associated with glutathione peroxidase from column fractions was found to be about 2.5 times greater in New Zealand residents than in Oregon residents. The chemical form of selenium in the proteins was investigated and showed that selenium is present in glutathione peroxidase and selenoprotein P as selenocysteine and in stoichiometric amounts, whereas that in haemoglobin and albumin is in the form of selenomethionine and on a random basis.¹⁷ To assess further the influence of selenium status, blood was collected from men living in areas of China with deficient, adequate and excessive levels of selenium.¹⁸ There was an expected increase in plasma and erythrocyte selenium concentrations with each increase in selenium status, but glutathione peroxidase did not increase beyond the level attained at adequate selenium status.

These observations were consistent with the earlier described observations of urinary excretion and retention of selenium after supplementation with the inorganic form and the organic form selenomethionine. Thus dietary forms of selenium were having a marked influence on the distribution of selenium in erythrocyte and plasma protein fractions, and although whole blood selenium levels have been useful in research, they do not provide the whole picture of selenium bioavailability.¹⁷ These studies illustrate two points which have major implications for assessment of selenium status in human blood.

(i) The lower the blood selenium concentration, the greater is the percentage of total erythrocyte selenium associated with glutathione peroxidase, which explains the more significant correlations between selenium content and glutathione peroxidase activity in New Zealand subjects. The same applies to plasma selenium. Lower correlations with plasma are probably due to the third protein selenoprotein P, which complicates the issue of relationships between selenium and selenoprotein levels.

(ii) The bioavailability of selenium and its distribution in the organism depend to a large extent on its chemical form in the diet. Selenomethionine follows the same pathways as methionine and is incorporated into body tissues in a non-specific and unregulated manner, whereas selenite and other forms of selenium are incorporated into a body pool which is used exclusively for functional forms of selenium and appears to be under homeostatic regulation. Selenomethionine is thus retained in tissues and tissue proteins to a much greater extent than selenite and may in fact accumulate there, and increases in tissue concentration associated with selenomethionine intake will be mainly caused by this non-specific incorporation into many proteins. On the other hand, intake of other forms such as selenate or selenocysteine will be excreted in the urine if in excess. At high dietary intakes of selenomethionine the tissue selenium content therefore cannot be taken as a measure for the levels of the biologically active selenoproteins.¹⁹ This has implications also with respect to evaluating the association between selenium and diseases, as discussed later.

It will be of interest to determine the extent to which selenium in selenomethionine can be mobilised and used again for incorporation into the biologically active selenoproteins and what factors determine this re-utilisation, and whether it acts as a storage form of selenium for periods of sub-optimal supply.²⁰

Chemical form of selenium in foods

These early studies raised the question of whether any of these forms were in fact normal dietary constituents. We still know comparatively little about the forms in food. It is believed that the major form in cereals is probably the selenoamino acid selenomethionine,²¹ and because selenocysteine is the active form of selenium in functional selenoproteins in the mammalian body, it is assumed that this is the form of selenium in animal foods. However, the extent of occurrence of inorganic forms or other forms of selenium is not at all clear. This has made conclusions about the bioavailability of selenium in different foods difficult to assess.

Rat studies indicate differences in bioavailability of selenium in foods, ranging from very low in mushrooms (5%) to high in Brazil nuts (124%) in comparison with selenite (100%).²² To learn a little about the bioavailability of food selenium we carried out long-term supplementation studies with high-selenium wheat bread and fish selenium and compared them with selenomethionine and selenite. As expected, wheat selenium behaved much like selenomethionine in terms of urinary excretion and blood selenium concentrations,²³ whereas fish selenium appeared to be less readily absorbed than selenomethionine, but retention of the total intake was similar.²⁴ Bioavailability for glutathione peroxidase activity appeared to be similar for all forms. Meltzer et al.²⁵ reported similar results when comparing the bioavailability of wheat and fish selenium in Norwegians, whereas van der Torre et al.²⁶ have shown that the bioavailability of wheat and meat selenium was similar with respect to activities of glutathione peroxidase. The effect of fish intake on plasma glutathione peroxidase and selenoprotein P was assessed by Huang et al.,²⁷ who showed that with increasing intake of fish, plasma selenium increased but there was no corresponding increase in plasma glutathione peroxidase or selenoprotein P. This may be related to the chemical form of selenium in fish, which appears to vary in different
high levels of selenium. This has been termed by Behne and co-workers in Germany, among species of animal and also among tissues in the same species.26 We know very little about most of these at present. In the process of incorporation of selenium into selenoproteins, the unique selenocysteine-inserting tRNA species tRNAsec is charged with selenocysteine, which is then converted by selenocysteine synthetase into selenocysteine. The selenocysteyl-tRNA recognizes specific UGA codons in mRNA to insert selenocysteine into the primary structure of selenoproteins.27 Those which have been characterised and for which functions are known include four species of glutathione peroxidase (cellular or classic, extracellular or plasma, phospholipid hydroperoxide glutathione peroxidase and gastrointestinal), selenoprotein P, of which there may also be many forms,30 selenoprotein W, found in muscle, iodothyronine deiodinase, thioredoxin reductase, a sperm mitochondria selenoprotein and a number of binding proteins.

Any of these selenoproteins could potentially be used for the assessment of selenium status. However, there are differences among species of animal and also among tissues in the same species. tRNAsec is charged with selenocysteine, which is then converted by selenocysteine synthetase into selenocysteine. The selenocysteyl-tRNA recognizes specific UGA codons in mRNA to insert selenocysteine into the primary structure of selenoproteins.27 Those which have been characterised and for which functions are known include four species of glutathione peroxidase (cellular or classic, extracellular or plasma, phospholipid hydroperoxide glutathione peroxidase and gastrointestinal), selenoprotein P, of which there may also be many forms,30 selenoprotein W, found in muscle, iodothyronine deiodinase, thioredoxin reductase, a sperm mitochondria selenoprotein and a number of binding proteins.

The first of these selenoproteins to be isolated and identified was glutathione peroxidase, and as in other functional proteins the selenium is an essential component and is always present as selenocysteine in the active site. Since its discovery, glutathione peroxidase has been used to assess selenium status in countries of low or marginal selenium status such as New Zealand but it is not suitable at higher selenium status. Glutathione peroxidase appears to be at the bottom of the hierarchy of importance, and is therefore the first to decrease in selenium deficiency, thus in a more marginal deficiency this enzyme will be decreased but other important selenoproteins such as iodothyronine deiodinase will be in the normal range.

Not only is there preferential incorporation into certain selenoproteins, but there is also a preferential distribution among various tissues. During periods of insufficient selenium intake the brain and endocrine organs are preferentially supplied and, within these tissues, selenium-containing proteins other than glutathione peroxidase. The maintenance of selenium levels in tissues such as the liver, heart, skeletal muscle and red blood cells is lowest in this hierarchy, and this may explain why pathological effects of selenium deficiency first appear in these tissues. In these tissues also the incorporation into most of the other selenoproteins has priority over glutathione peroxidase.20 The next selenoprotein to decrease as a result of insufficient selenium intake appears to be selenoprotein P, indicating that more selenium is conserved for the biosynthesis of selenoprotein P than that of glutathione peroxidase.32 Studies in rats indicate that the level of mRNA of selenoprotein P was less affected by selenium deficiency than those of other selenoproteins,33 and when selenium is injected into selenium-deficient rats, the selenoprotein P concentration increases before that of glutathione peroxidase.34 Similarly, selenium depletions resulting in reduced cytosolic glutathione peroxidase had no effect on phospholipid glutathione peroxidase mRNA.35 These observations indicate differential regulation of these selenoproteins. Burk et al.36 recently illustrated the effect of different levels of selenium status on levels of the selenoproteins glutathione peroxidase, selenoprotein P and selenomethionine in human plasma. The last form was the first to show a decrease with decreasing selenium status. As selenium status becomes marginal, glutathione peroxidase begins to fall before selenoprotein P. In severe deficiency, glutathione peroxidase falls to 1% in rat plasma, but selenoprotein P to only 10%.34

Looking at this effect from another point of view, Behne et al.19 pointed out that only a small percentage of the tissue selenium is present in the selenoenzyme iodothyronine deiodinase, and therefore changes in the level of this protein through some factor other than selenium status will not be reflected by the total element content. In the investigation of the essential effects to trace elements, misleading results can, therefore, often be obtained if only the total element content is taken into consideration.19 This complexity means that we can no longer use levels of total selenium or of one selenoprotein for assessing nutritional status or of just one analytical technique. As we learn more about the biochemical and clinical importance of other selenoproteins such as selenoprotein P, phospholipid hydroperoxide glutathione peroxidase and iodothyronine deiodinase, and as routine biochemical assays of these selenoproteins become available, these will become the means for assessing functional selenium status in the future, whereas the measurement of the trace element levels will become of lesser importance.

**Factors which affect speciation of selenium compounds**

A number of other factors besides chemical form of dietary selenium and size of supplemental intake may influence the distribution of trace element-containing chemical species before and after sample collection, as outlined by Gardiner.37 Some of these may be physiological which are of interest in elucidating the metabolism of this trace element.

(i) Dietary factors other than the size and chemical form of the element may affect the distribution of selenium among tissues, for example methionine intake influences absorption and distribution of selenium in rats.38

(ii) The tissue distribution of many of the selenoproteins differs with species, for example there are high levels of selenoprotein W in sheep heart but it is almost absent from rat heart.39 Differences in distribution in tissues in the same species have also been mentioned previously.

(iii) The selenium status of an animal may also influence speciation of this trace element. Specific incorporation of radioactively labelled selenium into proteins is more effective the lower the selenium status of the animals and the smaller the amount of tracer administered.40 We have also shown that previous selenium supplementation affects the retention of enriched stable isotopes of selenium in human subjects in New Zealand consuming diets low in selenium.41

(iv) Robberecht and Deelstra42 discussed various factors that influence blood selenium concentrations such as age, gender, smoking, drinking and race. We can document some of these effects but we do not know to what extent any of these factors affect individual selenoproteins and whether they affect different proteins in different ways.
(v) The physiological state of the body such as lactation and pregnancy may also influence selenium metabolism and speciation.

**Implications for selenium requirements and recommended dietary intakes**

Because the bioavailability and distribution of selenium among functional proteins are dependent on the amounts and chemical forms of the trace element in the diet, information about these relationships is of special interest with regard to selenium requirements and the determination of optimum selenium intakes. The incorporation of selenium into most of the other selenoproteins has priority over that of plasma and cytosolic glutathione peroxidase. This means that the requirements for selenium which have been calculated based on optimum plasma glutathione peroxidase activity must also cover the amounts needed for normal levels of the other biologically important selenium compounds. However, if it could be shown that optimisation of glutathione peroxidase is not necessary for health then the present recommended daily allowances may in fact be higher than necessary. The World Health Organization recommended intakes are based on two-thirds maximum glutathione peroxidase activity and their ‘normative requirement estimates’ are considerably lower than the US recommended dietary allowance and the Australian recommended nutrient intakes. It has yet to be determined whether this lower level of intake is sufficient for optimum levels of other selenoproteins such as selenoprotein P, deiodinase and phospholipid hydroperoxide glutathione peroxidase.

**Implications for relationships between selenium and health and disease**

As we have seen, total trace element composition is less important than levels of individual selenoproteins and in fact may be misleading. Low concentrations of selenium in blood have been implicated as a risk factor for numerous different diseases including cardiovascular disease, cancer and rheumatoid arthritis. However, in the light of all the recent developments discussed here, we should question the conclusions drawn from research which reports only total blood selenium concentrations and attempts made to correlate these with some clinical condition.

One illustration of this pointed out by Burk relates to the non-specific incorporation of selenomethionine into tissue proteins, which has implications with respect to evaluating the association between selenium and cancer prevention. Epidemiological studies have shown a negative correlation between selenium status and the incidence of some types of cancer. However, selenium in cereal and vegetable products is most likely to be present in the form of selenomethionine. Therefore, individuals who consume large amounts of vegetables and cereals, particularly in areas of higher soil selenium, may have higher blood selenium levels than those who do not. The selenium is not present necessarily in a functional form and therefore the high selenium status might be misleading. It may in fact be some other factor associated with high vegetable intake, such as carotene, which is the protective factor.

Another illustration relates to differences in the distribution of selenium in selenoproteins among species and tissues within the same species. There are high levels of the selenoprotein W in sheep heart muscle but it is almost absent from rat heart. It was suggested that this may relate to the difference between the two species in syndromes of selenium deficiency which results in cardiac damage in sheep but not in rats. Hence this selenoprotein appears not to be important at least in the rat heart, and there are a number of other examples of such differences among species and tissues.

The final illustration comes again from the work of Burk et al., who showed that in children with cirrhosis, levels of selenoproteins in plasma change differently in relation to the severity of the disease. Selenomethionine-containing proteins such as albumin are depleted first, glutathione peroxidase in fact increases and selenoprotein P falls only in very ill patients. Hence we need to investigate individual species in relation to physiological and metabolic processes associated with health and disease, and specification showing the patterns of changes in selenoproteins may throw some light on what is happening during the process and development of a disease.

In conclusion, in order to be able to assess the selenium status as accurately as possible, we need monitors which reflect an insufficient selenium intake in terms of availability for biochemical function, but are also affected in the same way as the body pools when the element is taken up in adequate and large amounts. We can no longer rely on total tissue selenium concentrations to study the biological effects of selenium, but must consider levels of a number of functional selenoproteins, their distribution in tissues and their response to deficient, adequate and toxic levels of dietary selenium.

**References**
