Review article

Selenium and its relationship to cancer: an update†

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Selenomethionine (Semet) is the major seleno-compound in cereal grains and enriched yeast whereas Se-methylselenocysteine (SeMCYS) is the major seleno-compound in Se-accumulator plants and some plants of economic importance such as garlic and broccoli exposed to excess Se. Animals can metabolize both Semet and SeMCYS. Epidemiological studies have indicated an inverse relationship between Se intake and the incidence of certain cancers. Blood or plasma levels of Se are usually lower in patients with cancer than those without this disorder, but inconsistent results have been found with toenail-Se values and the incidence of cancer. There have been eight trials with human subjects conducted on the influence of Se on cancer incidence or biomarkers, and except for one, all have shown a positive benefit of Se on cancer reduction or biomarkers of this disorder. This is consistent with about 100 small-animal studies where Se has been shown to reduce the incidence of tumours in most of these trials. Se-enriched yeast is the major form of Se used in trials with human subjects. In the mammary-tumour model, SeMCYS has been shown to be the most effective seleno-compound identified so far in reduction of tumours. Several mechanisms have been proposed on the mechanism whereby Se reduces tumours. Even though SeMCYS was shown to be the most effective seleno-compound in the reduction of mammary tumours, it may not be the most effective seleno-compound for reduction of colon tumours.

Selenium: Cancer

Not many elements have such an interesting history as Se. The present introduction is written to indicate the wide use of Se in addition to its beneficial effects against certain cancers. One property of Se has had a profound influence on humanity, namely its photoconductivity. As early as 1884 a television system was devised relying on mechanical sequence of light values to corresponding electrical values. After transmission to a receiver, a lamp reproduced the sequence of light values. In 1926, an investigator by the name of Baird demonstrated the electric transmission of moving pictures in half-tones. It is said that in his training, Baird had devised an improved Se cell and that this achievement led him to develop a very early form of 'true' television (Smith-Rose, 1926). In addition, Se plays a fundamental role in the process of xerography. It is indeed difficult to imagine present-day life in a technology-driven country with neither copying machines nor television, in which one technology is still relying on Se and the other one profoundly influenced by Se in its development. It goes without saying that few elements have had such an influence, whether for better or for worse, on human lives.

Se was discovered by Berzelius in 1817 and was named after the moon goddess. It has found many uses in industry, namely in the manufacture of ceramics and glass, in photo-electric cells and xerography as noted earlier, in semiconductors and the vulcanization of rubber, and a few in agriculture, such as the use of seleno-diethyldithiocarbamate as a fungicide and in fertilizer to increase the Se content of plants in order to protect grazing animals against deficiency, and in medicines such as selenium sulfide, which is use as in a shampoo for treatment of tinea versicolor. More importantly, the medical aspects also include a possible role as a protective agent in neurotoxicity (Imam & Ali, 2000) and in prevention of cancer, which is the subject of the present review.

Se has come full circle in two aspects. Initially the only concern for this element was its toxicity. It is now recognized as an important essential element. It was once thought to promote cancer, but it is now realized that this element will prevent certain types of cancer. A discussion of the anti-carcinogenic function of Se is the purpose of the present review. It should be pointed out that the concentration of Se in the earth's crust is less than that for Au. Thus, we are dealing with an element rarer than Au.
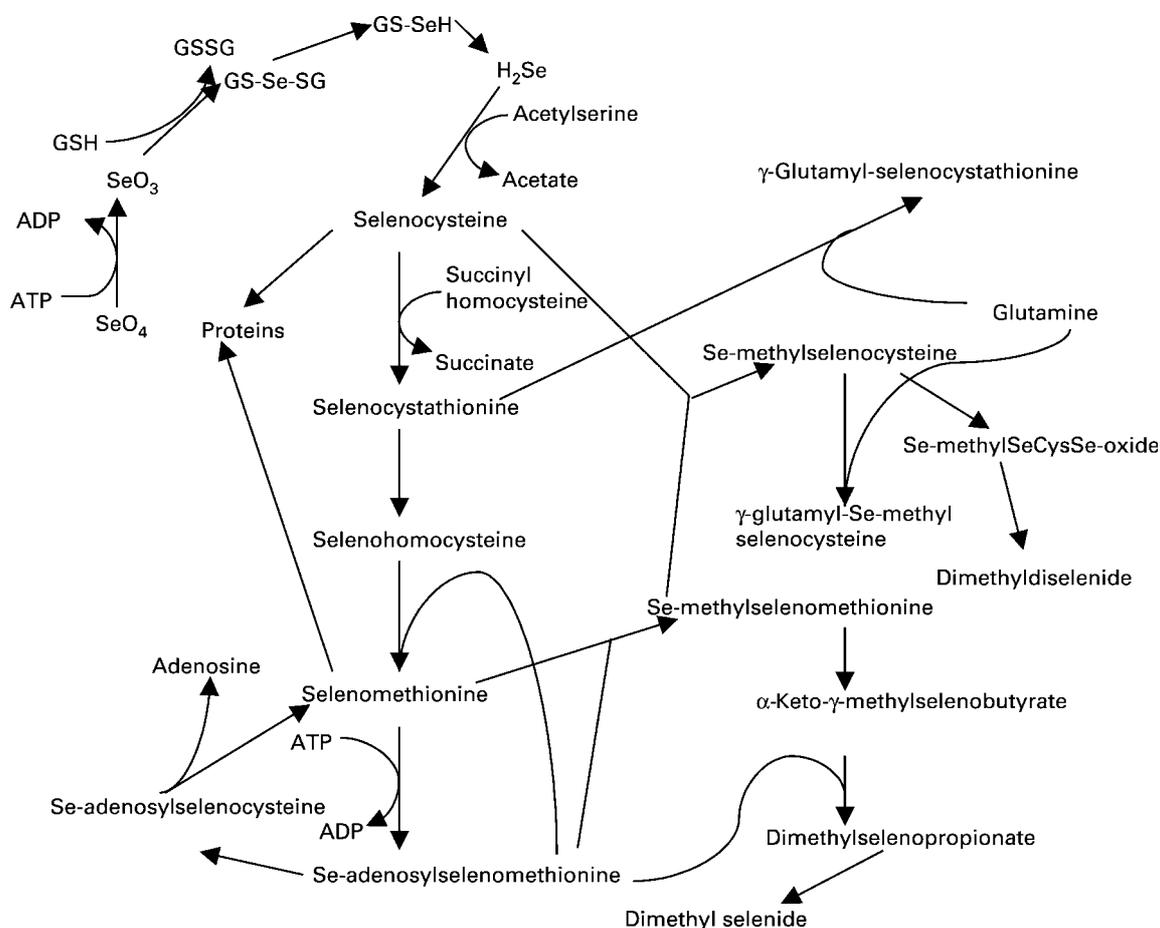

Selenium

The chemical and physical properties of Se are very similar to those of S (Combs & Combs, 1986a). The two elements have similar outer valence-shell electronic configurations and atomic sizes, and their bond energies, ionization potentials and electron affinities are virtually the same. Despite these similarities, the biochemistry of Se and S differ in at least two respects that distinguish them in biological systems. First, in biological systems Se compounds are metabolized to more reduced states, whereas S compounds are metabolized to more oxidized states. The second important difference in the chemical behaviours of these elements is in the acid strengths of their hydrides. The hydride H_2Se is much more acidic than is H_2S. This difference in acidic strengths is reflected in the dissociation behaviours of the selenohydryl groups of selenocysteine and the sulfhydryl groups on cysteine. Hence, while thiols such as cysteine are predominantly protonated at physiological pH, the selenohydryl groups of selenols such as selenocysteine are predominantly dissociated under the same conditions. These differences between Se and S are the reasons seleno-compounds are usually 600 times more effective than their S analogues against tumours (Ip & Ganther, 1992a).

Seleno-compounds in plants

The metabolism of seleno-compounds in plants as well as the species of Se-accumulator plants have been summarized by Whanger (1989, 2003) and Terry *et al.* (2000). The metabolic pathways for Se metabolism are presented in Fig. 1. Recent results indicate that the seleno-compounds present in plants may have a profound effect upon the health of animals and human subjects. It is now known that the total Se content cannot be used as an indication of its efficacy, but knowledge of individual seleno-compounds is necessary to fully assess the significance. Thus, speciation of the seleno-compounds has moved to the forefront. Since animals and man are dependent upon plants for their nutritional requirements, this makes the types of seleno-compounds in plants even more critical.

Selenate is reduced to selenide by a number of steps that involved reduced glutathione. Selenide reacts with *O*-acetylserine to form selenocysteine in a manner directly analogous to S metabolism (Ng & Anderson, 1979). The S-amino acid cysteine is the starting point for a series of reactions that lead to the synthesis of methionine and it has been postulated, mostly due to lack of experimental evidence to the contrary, that selenocysteine is also metabolized by this same pathway. Se enters the food chain through incorporation into plant proteins, mostly as selenocysteine

Fig. 1. Proposed pathways for the metabolism of selenium in plants. Cys, cysteine; GSH, reduced glutathione; GSSG, oxidized glutathione; GS-Se-SG, selenodiglutathione. (From Whanger, 2003.)

and selenomethionine (Semet) at normal Se levels. However, with elevated Se levels, Se-methylselenocysteine (SeMCYS) can be the predominant seleno-compound. As many as eight other seleno-compounds have been identified in plants, but their concentrations are usually very low except at high Se levels. Indicator plants (called Se-accumulators) can accumulate extremely large amounts of Se, ranging from 1000 to 10 000 μg Se/g, because they synthesize mostly non-protein selenoamino acids (Brown & Shrift, 1981). As much as 80 % of the total Se in some accumulator plants is present as SeMCYS, which until recently was thought to be absent in non-accumulator plants.

The Se content of plants is dependent upon the region of growth (Whanger, 1989; Terry *et al.* 2000). Vegetables such as rutabagas, cabbages, peas, beans, carrots, tomatoes, beets, potatoes and cucumbers contained a maximum of 6 μg Se/g even when grown on seleniferous soils. Vegetables such as onions and asparagus may accumulate up to 17 μg Se/g when grown on these types of soils. Plants can synthesize organic Se compounds including Semet from inorganic Se (Burnell & Shrift, 1977). Because of the uneven global distribution of Se, disorders of both Se deficiency and Se excess are known. As an example, China has regions with both the lowest and the highest Se-containing soil in the world (Yang *et al.* 1989*a*,*b*). Plants of economic importance do not have a Se requirement for growth and thus plant-Se is important for the health of animals including man.

Plants which contain deficient levels of Se are found in the Pacific north-west, upper mid-west, and the New England states and along the Atlantic coast of the USA. In other parts of the country such as ND and SD, CO and western NE plants may contain high levels of this element. Information on the distribution of Se on a worldwide basis has been presented by Oldfield (1999) and Combs (2001). There are Se-deficient areas in Australia along the coasts of the states of Queensland, New South Wales, Victoria, South Australia, Western Australia and the interior of Tasmania. The only reported area of high levels of Se is in the northern part of Queensland. The most widely Se-deficient area in New Zealand is along the east coast of the South Island and this area extends almost to the middle of the island. The only region of Se deficiency in the North Island is a small area in the middle part of this area of New Zealand. Low-Se areas have been reported in various countries in Europe, including Scotland, Denmark, Norway, Sweden, Finland, UK, Spain, Greece, Turkey and the Balkans, but there does not appear to be any areas of excess Se in these countries. Based on the results obtained thus far, no very-low-Se or high-Se areas have been found in Russia, but there are vast territories that remain to be charted. China has the lowest-Se areas in the world as well as the highest. A band of Se-deficient soil extends from the north-east to the south-central part of this country. Interestingly, there is a high-Se area in the region (Hubei province of Enshi county) adjacent to the deficient area of Sichuan Province. In fact, the first world site to record Se deficiency in humans (Keshan disease) was in China. There does not appear to be any concern about Se deficiency in Japan. Information on Se status in South America is meagre.

There are known to be seleniferous areas in Venezuela, mostly in the Andes Mountains. Some information is available to indicate Se-deficient areas in Argentina. Likewise, information on Se status in Africa is meagre, but there are apparently some low-Se areas. Interestingly, AIDS appear to be more prevalent in areas of Africa with low-Se status (Foster, 2002).

Although the data are lacking, synthesis of the non-protein selenoamino acids by plants probably occurs along pathways normally associated with S metabolism. Conversion of selenocysteine to SeMCYS in accumulators has been shown to involve the transfer of a methyl group from S-adenosylmethionine analogous to the synthesis of *S*-methylcysteine (Neuhierl *et al.* 1999). Even though the primary source of Se in soil is inorganic (mostly selenate), *Astragalus* accumulators have been shown to synthesize SeMCYS when supplied with Semet (Chen *et al.* 1970). The ability of the accumulators to exclude selenoamino acids from proteins has been suggested as a reason for their Se tolerance. Similar mechanisms apparently operate in Se-enriched plants such as garlic, broccoli, onions and wild leeks, where the non-protein selenoamino, SeMCYS, is the predominant form present (Whanger, 2002).

The seleno-compounds present in enriched plants have been summarized by Whanger (2002). Most of the Se in enriched wheat grain (Olson *et al.* 1970), maize and rice (Beilstein *et al.* 1991), and soyabeans (Yasumoto *et al.* 1984) is present as Semet. Semet is also the predominant form of Se in Se-enriched yeast (Ip *et al.* 2000*a*). Se-enriched yeast is the most common source of Se available commercially (Schrauzer, 2000). The selenoamino acid, Semet, is also available to the public. The major form of Se in Se-enriched garlic (Ip *et al.* 2000*a*), onions (Cai *et al.* 1995), broccoli florets (Cai *et al.* 1995), broccoli sprouts (Finley *et al.* 2001) and wild leeks (Whanger *et al.* 2000) is SeMCYS.

Seleno-compounds in animals

A brief metabolic pathway for Se metabolism in animals has been presented by Ip (1998) and the seleno-compounds in animal tissue have been summarized by Whanger (2002). The metabolic pathways for Se in animals are shown in Fig. 2. Organic Se such as Semet or inorganic Se can be converted to a common intermediate, H_2Se.

There are two possible pathways for the catabolism of Semet. One is the transsulfuration pathway via selenocystathionine to produce selenocysteine, which in turn is degraded to H_2Se by the enzyme β-lyase. The other pathway is the transamination–decarboxylation pathway (Mitchell & Benevenga, 1978). It was estimated that 90 % of methionine is metabolized through this pathway and thus could be also the major route for Semet catabolism. SeMCYS is the predominant seleno-compound formed in Se-enriched garlic at relatively low concentrations, but γ-glutamyl-SeMCYS is the predominant form at high Se concentrations (Dong *et al.* 2001). Even though this glutamyl derivative may be the predominant form, it is hydrolysed in the intestinal tract and the absorbed SeMCYS cleaved by a lyase to form methylselenol (Dong *et al.* 2001). Thus, this glutamyl derivative is
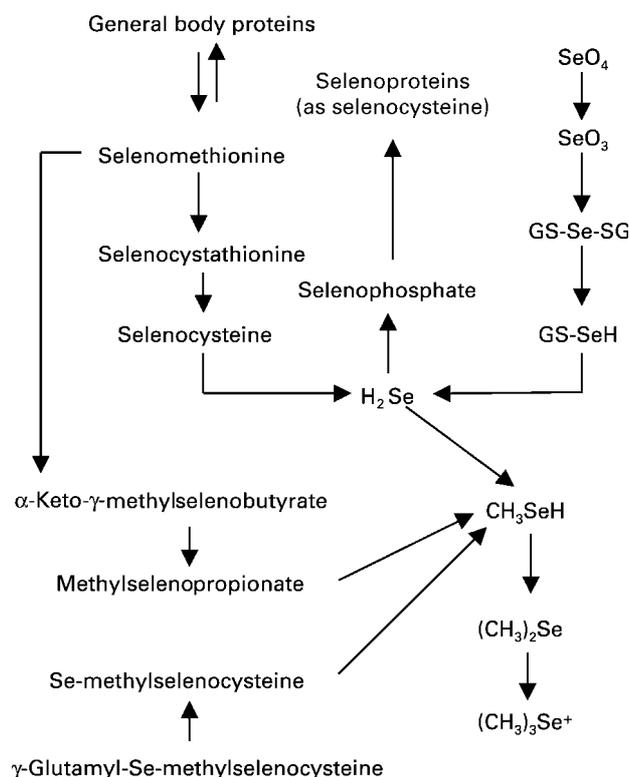
Fig. 2. Proposed pathways for the metabolism of selenium in animals. GSH, reduced glutathione; GSSG, oxidized glutathione; GS-Se-SG, selenodiglutathione. (From Whanger, 2003.)

metabolized like SeMCYS at the tissue level. SeMCYS is converted to methylselenol directly when cleaved by β-lyase, and unlike Semet it cannot be incorporated non-specifically into proteins. Since SeMCYS can be converted directly to methylselenol, this explains why it is more efficacious than other forms of Se in cancer prevention.

When rats are injected with selenite, the majority of the Se is present in tissues as selenocysteine (Olson & Palmer, 1976; Beilstein & Whanger, 1988). As expected, no Semet was found under the conditions of these studies. In contrast to plants, there is no known pathway in animals for synthesis of Semet from inorganic Se, and thus they must depend upon plant or microbial sources for this selenoamino acid. However, animals can convert Semet to selenocysteine. One day after injection of Semet there is about three times as much Semet as selenocysteine in tissues, but ≤5 d afterwards the majority (46–57 %) of the Se is present as selenocysteine (Beilstein & Whanger, 1986, 1988).

A total of twenty-five selenoproteins have been identified in eukaryotes (Gladyshev, 2001; Kryukov *et al.* 2003). A table of the characteristic of all twenty-five selenoproteins has been assembled by Kryukov *et al.* (2003). These selenoproteins have been subdivided into groups based on the location of selenocysteine in selenoprotein polypeptides. The first group is the most abundant and includes proteins in which selenocysteine is located in the N-terminal portion of a relatively short functional domain. These include the four glutathione peroxidases (GPX) and selenoproteins P, Pb, W, W2, T T2 and BthD (from *Drosophila*). The

second group of eukaryotic selenoproteins is characterized by the presence of selenocysteine in C-terminal sequences. These include the three thioredoxin reductases and the G-rich protein from *Drosophila*. Other eukaryotic selenoproteins are currently placed in the third group that consists of the three deiodinase isozymes, selenoproteins R and N, the 15 kDa selenoprotein and selenophosphate synthetase. The four GPX are located in different parts of tissues and all detoxify H_2O_2 and fatty acid-derived hydroperoxides to various degrees and thus are considered antioxidant selenoenzymes. The three deiodinases convert thyroxine to triiodothyronine, thus regulating thyroid hormone metabolism. The thioredoxin reductases reduce intramolecular disulfide bonds and, among other reactions, regenerate vitamin C from its oxidized state. These reductases can also affect the redox regulation of a variety of factors, including ribonucleotide reductase, the glucocorticoid receptor and the transcription factors (Holmgren, 2001). Selenophosphate synthetase synthesizes selenophosphate, which is a precursor for the synthesis of selenocysteine (Mansell & Berry, 2001). The functions of the other selenoproteins have not been definitely identified.

Se is present in all eukaryotic selenoproteins as selenocysteine (Gladyshev, 2001). Semet is incorporated randomly in animal proteins in place of methionine. By contrast, the incorporation of selenocysteine into proteins known as selenoproteins is not random. Thus, in contrast to Semet, selenocysteine does not randomly substitute for cysteine. In fact, selenocysteine has its own triplet code (UGA) and is considered to be the twenty-first genetically coded amino acid. Interestingly, UGA has a dual role in the genetic code, serving as a signal for termination and also a codon for selenocysteine. Whether it serves as a stop codon or encodes selenocysteine depends upon the location of what is called the selenocysteine insertion sequence (Mansell & Berry, 2001). The selenocysteine insertion sequences (seven so far) for the various selenoproteins have been presented by Kryukov *et al.* (2003).

Epidemiological studies

There have been a number of epidemiological studies in the USA and throughout the world on the relationship between Se and cancer. Shamberger & Frost (1969) reported that the Se status of human subjects might be inversely related to the risk of some kinds of cancer. Two years later, Shamberger & Willis (1971) indicated in more extensive studies that the mortality due to lymphomas and cancers of the gastrointestinal tract, peritoneum, lung and breast were lower for men and women residing in areas of the USA that have high concentrations of Se in forage crops than those residing in areas with low Se content in the forages. Those studies were supported by a later analysis of colo-rectal cancer mortality using the same forage data (Clark *et al.* 1981). A twenty-seven country comparison revealed that total cancer mortality rate and age-corrected mortality due to leukaemia and cancers of the colon, rectum, breast, ovary and lung varied inversely with estimated Se intake per capita Schrauzer *et al.* (1977). Similar results were also reported

in China, a country where Se intakes range from deficient to toxic levels (Yu *et al.* 1985).

Lower Se levels were found in serum collected from US subjects 1–5 years before diagnosis of cancer as compared with those who remained cancer-free during this time (Willett *et al.* 1983). That association was strongest for gastrointestinal and prostatic cancers.

Evidence that low serum Se is a prediagnostic indicator of higher cancer-risk was subsequently shown in studies conducted in Finland (Salonen *et al.* 1984) and Japan (Ujiie *et al.* 1988). In further case–control studies, low serum or plasma Se concentrations were found to be associated with increased risk of thyroid cancer (Glattre *et al.* 1989), malignant oral cavity lesions (Toma *et al.* 1991), prostate cancer (Brooks *et al.* 2001), oesophageal and gastric cancers (Mark *et al.* 2000), cervical cancer mortality rates (Guo *et al.* 1994) and colo-rectal adenomas (Russo *et al.* 1997). A 10-year prospective study of Se status and cancer incidences indicated that initial plasma Se concentration was inversely related to subsequent risks of both non-melanoma skin cancer and colonic adenomatous polyps (Clark *et al.* 1993). Patients with plasma Se levels <128 ng/ml (the average normal value) were four times more likely to have one or more adenomatous polyps. An 8-year retrospective case–control study in MD, USA, revealed no significant association of serum Se level and cancer risk at sites other than the bladder (Helzlsouer *et al.* 1989), but those with low plasma Se levels had a 2-fold greater risk of bladder cancer than those with high plasma Se. In a study with Dutch patients the mean Se levels were significantly less than that of control values in men, but no differences were found in plasma Se levels between healthy control women and those with cancer (Kok *et al.* 1987). No significant associations in three other studies were found between serum Se concentration and risk of total cancers (Coates *et al.* 1988) or cancers of the lungs, stomach or rectum (Nomura *et al.* 1987; Kabuto *et al.* 1994). In other work, significant increases of urinary Se excretion were found in Mexican women with cervical uterine cancer compared with control values (Navarrete *et al.* 2001).

In four studies low toenail-Se values were associated with higher risks of developing cancers of the lung (van den Brandt *et al.* 1993*a*), stomach (van den Brandt *et al.* 1993*b*), breast (Garland *et al.* 1995) and prostate (Yoshizawa *et al.* 1998). In contrast, in four other studies no significant differences were found between cancer cases and controls (Noord *et al.* 1987; Hunter *et al.* 1990; van't Veer *et al.* 1990; Rogers *et al.* 1991). It has been suggested that the reason for these studies not showing a relationship is that the Se intakes of most of the subjects tested were below that necessary for protection (Schrauzer, 2000). Obviously these results indicate that many factors must be taken into consideration when evaluating plasma and toenail-Se concentrations in relation to cancer incidence.

Trials with human subjects

In spite of advances in diagnosis and treatment, cancer continues to be a major health burden. With the fear associated with diagnosis of cancer, it is not surprising that the public may have intense interest in easily implemented measures, such as dietary modification or use of vitamin and trace element supplementation for cancer prevention. Promising results have been obtained indicating that Se supplementation is effective in the reduction of certain cancers in human subjects, as discussed in the present section.

There have been eight trials conducted on the effects of Se supplementation on the incidence of cancer or biomarkers in human subjects and all of them have shown positive effects of Se. Five of these were conducted in China and one each in India, Italy and the USA. The first intervention trial to prevent cancer with Se in human subjects was conducted in Qidong, a region north of Shanghai, China, where there is a high incidence of primary liver cancer. Subjects were given table salt fortified with 15 μg Se as sodium selenite/g, which provided about 30–50 μg Se/d for 8 years (Yu *et al.* 1991, 1997). This resulted in a drop of the primary liver cancer incidence to almost one-half (27·2 per 100 000 population *v.* 50·4 per 100 000 population consuming ordinary salt). Upon withdrawal of Se from the treated group, the primary liver cancer incidence began to rise. In a separate study, risk populations receiving selenite salt as a source of Se also showed a significant reduction in the incidence rate of viral infectious hepatitis, a major predisposing primary liver cancer risk factor in this region (Yu *et al.* 1989). The Se-fortified salt was distributed to a general population of 20 800 persons. People in six neighbouring townships served as controls and were given normal table salt.

In a second trial, members of families at risk of primary liver cancer were either given 200 μg Se/d in the form of high-Se yeast or a placebo (Yu *et al.* 1997). During the 2-year study period, 1·26 % of the controls developed primary liver cancer *v.* 0·69 % in those given Se-enriched yeast. This difference was significantly different ($P<0·05$). Furthermore, of 226 hepatitis B surface antigen carriers, seven of 113 subjects in the placebo group developed primary liver cancer during 4 years as opposed to no cases in those taking Se-enriched yeast.

A third trial on the effects of Se on cancer was also conducted in China and included 3698 subjects. This intervention trial was conducted from 1984 to 1991 in Linxian, China, a rural county in Henan Province, where the mortalities from oesophageal cancer are among the highest in the world (Blot *et al.* 1993). The results indicated that a treatment containing Se (50 μg Se/d as Se-enriched yeast plus vitamin E and β-carotene) produced a modest protective effect against oesophageal and stomach cancer mortality among subjects in the general population (Taylor *et al.* 1994; Blot *et al.* 1995). Probably the reason for only a modest reduction of cancer by Se is that only 50 μg/d were given in contrast to other studies where up to 200 μg/d were given.

In the fourth trial a total of 29 584 adults in China were used to evaluate the effects of vitamins and minerals on cancer (Blot *et al.* 1993). Four combinations of nutrients were evaluated in a factorial design: (1) retinol and Zn; (2) riboflavin and niacin; (3) vitamin C and Mo; (4) β-carotene, vitamin A and Se (50 μg Se/d as enriched yeast). No significant effects were associated with the first three

supplement regimens, but total mortality and cancer mortality were significantly lower (relative risk 0·87, 95 % CI 0·75, 1·00) among those who received the combination of β-carotene, vitamin E and Se. The reduction was greater for stomach than oesophageal cancers (but not significantly so) and began to be apparent about 2 years into the supplementation (Blot, 1997). Rates of lung cancer, the third most common cancer, were only about half as high among those receiving v. those not receiving β-carotene, vitamin E and Se.

In the fifth study of human subjects, 3318 subjects with cytological evidence of oesophageal dysplasia were randomly assigned to receive daily supplements of fourteen vitamins and twelve minerals with 50 μg Se as selenate or placebo for 6 years (Li et al. 1993). Doses of vitamins and minerals were two to three times US recommended daily allowances. Cumulative oesophageal or gastric cardia death rates were 8 % (relative risk 0·92, 95 % CI 0·67, 1·28) lower among individuals receiving supplements rather than the placebos, and were not statistically significant. Risk of total mortality was 7 % lower (relative risk 0·93, 95 % CI 0·75, 1·16). There are probably at least two reasons why a greater difference was not obtained between the supplemented and placebo groups. Animal studies indicate that Se is much more effective in the prevention of tumours rather than in reversing them (Ip, 1998), and thus the selection of subjects with evidence of oesophageal dysplasia may not been the best choice. Second, as noted by Li et al. (1993), 50 μg Se/d may not be sufficient to provide maximum protection.

In the study conducted in India, 298 subjects were studied. One-half of the subjects with pre-cancerous lesions in the oral cavity were supplemented with a mixture of four nutrients (vitamin A, riboflavin, Zn and Se (100 μg/d for 6 months and 50 μg as Se-enriched yeast/d in the final 6 months)) and compared with controls (also 149 patients) receiving placebos (Prasad et al. 1995). The frequency of micronuclei and DNA adducts were significantly reduced in the supplemented groups at the end of the 1-year study. The adducts decreased by 95 % in subjects taking Se with all categories of lesions and by 72 % in subjects without lesions. No such effects were noted in the placebo group.

In the Italian study subjects were given a mixture called 'Bio-Se' that provided 200 μg Se as L-Semet/d plus Zn and vitamins A, C and E for 5 years, and compared with those taking a placebo (Bonelli et al. 1998). A total of 304 patients participated in this study and the incidence of metachronous adenomas of the large bowel evaluated. Patients with previously resected adenomatous polyps were used in a randomized trial in which new adenomatous polyps were noted. The observed incidence of metachronous adenomas was 5·6 % in the group given the 'Bio-Se' mixture v. 11 % in the placebo group: this was statistically significant ($P<0.05$).

One of the most exciting clinical trials on Se and cancer in human subjects was conducted in the USA. In a simple experimental design (double-blind, placebo-controlled trial), 1312 older US subjects with histories of basal and/or squamous cell carcinomas of the skin were studied (Clark et al. 1996, 1998). The use of oral supplements of Se-enriched yeast (200 μg Se/d) did not affect the risk of recurrent skin cancers. However, such supplementation for a mean of 4·5 years significantly reduced the incidence of lung, colon and prostate cancers respectively by 46, 58 and 64 %. There were significant reductions in total cancer incidence in supplemented patients v. controls (relative risk 0·63, 95 % CI 0·47, 0·85) and incidences of lung, colo-rectal and prostate cancers (lung cancer incidence hazard ratio 0·56 (95 % CI 0·31, 1·01; $P=0.5$); prostate cancer incidence hazard ratio 0·35 (95 % CI 0·18, 0·65); colo-rectal cancer incidence hazard ratio 0·61 (95 % CI, 0·17, 0·90; $P=0.03$).

Restricting the analysis to the 843 patients with initially normal levels of prostate-specific antigen, only four cases were diagnosed with cancer in the Se-treated group but sixteen cases were diagnosed in the placebo group (hazard ratio 0·26; $P=0.009$) after a 2-year treatment lag (Clark et al. 1998). Even though Clark et al. (1996) did not observe any effect of Se on skin cancer in their study, the results strongly indicated that other types of skin disorders may be improved by Se. After 10 years of the trial, the trends were similar: the incidences of prostate, lung and colo-rectal cancers were reduced by 48, 29 and 53 % respectively (Duffield-Lillico et al. 2002). Se supplementation reduced the incidences of total cancer (hazard ratio 0·75, 95 % CI 0·58, 0·97) and prostate cancer (hazard ratio 0·48, 95 % CI 0·28, 0·80), but was not significantly associated with the incidences of lung cancer (hazard ratio 0·74, 95 % CI 0·44, 1·24) and colo-rectal cancer (hazard ratio 0·46, 95 % CI 0·21, 1·02). The protective effect of Se was confined to male subjects (hazard ratio 0·67, 95 % CI 0·50, 0·89) and was most pronounced in former smokers. Even though the reduction of prostate cancer was the only one that was statistically reduced, this is probably due to the small of number of patients remaining, particularly those with colo-rectal cancer (only nine in the Se-treatment group v. nineteen in the placebo group). The cancer incidence was evaluated according to the baseline plasma Se levels at the beginning of the study. The subjects with plasma Se in lower tertile (<105 and 105–122 ng Se/ml) had significantly lower incidences of cancer when supplemented with this Se-enriched yeast. However, those with plasma Se in the higher tertile (≥ 122 ng Se/ml) showed no effect of Se supplementation on the cancer incidence. This is in direct contrast to the epidemiological studies, where an inverse relationship in the incidence of cancer was observed with plasma Se levels and thus further evaluation of the data is paramount. However, an explanation could be that there is a threshold in plasma level above which further benefits would not be seen; thresholds may be near or above the plasma level achieved in most populations in the epidemiological studies.

The author is aware of at least two human trials to further evaluate the results of the US investigation: two in the USA (University of Arizona, and the SELECT trial at NCI; Klein et al. 2001)) and one planned in Europe (PRECISE; Rayman, 2000) when the money can be obtained.

Finally, in another trial topical application of Semet was effective in protecting against acute u.v. irradiation damage to the skin of human subjects (Burke et al. 1992a). Maximal protection appeared to be attained at concentrations between 0·2 and 0·5 g/kg. These results are consistent with some animal data. Hairless mice treated by topical

application of Semet (0·2 g/kg) or given drinking water with 1·5 μg Se as Semet/ml had significantly less skin damage due to u.v. irradiation (Burke *et al.* 1992*b*). This is consistent with an earlier study that indicated that dietary Se (1 μg/g) fed to mice significantly reduced the number of skin tumours induced by two carcinogenic chemicals plus croton oil (Shamberger, 1970).

Selenium and tumours in small animals

There have been more than 100 trials conducted with small animals on the relationship of tumour incidences to Se status (Combs & Combs, 1986*b*; Combs & Gray, 1998). Interestingly, the first evidence that Se may counteract tumours was presented in 1949 where the addition of Se to a diet for rats significantly reduced tumours caused by ingestion of an azo dye (Clayton & Bauman, 1949). Even these researchers ignored these results because of the negative image Se held at that time. The first evidence of the essentiality of Se was presented in 1957 (Schwarz & Foltz, 1957), at which time Se was considered a carcinogenic element. A number of reviews on Se and carcinogenesis in animals have been presented, and include those by Milner (1985), Ip & Medina (1987), Medina & Morrison (1988) and Whanger (1992). Two-thirds of the animal studies showed significant reductions by Se in the tumour incidence with one-half showing reductions of 50 % or more (Combs & Gray, 1998). In the majority of those studies Se as selenite was used, but that may not have been the most effective form to use (as noted later). Those results with animals and the epidemiological surveys showing a positive relationship between Se and cancer incidence were the main motivating factors for conducting trials with human subjects.

Use of tissue cultures to study selenium metabolism

The present research efforts are primarily focused on the mechanism of cancer reduction by Se, and tissue cultures have been used advantageously to study how tumours are reduced by this element. Research with mouse mammary epithelial cells indicates that the β-lyase-mediated production of a monomethylated Se metabolite (methylselenol) from SeMCYS is a key step in cancer chemoprevention by this agent (Ip *et al.* 2000*b*). In order for SeMCYS to be effective, cells must possess this β-lyase. One way to get around this is to use methylselenenic acid, which is even effective in cells without this lyase. Mouse mammary epithelial cells have low levels of the β-lyase. Interestingly, the distinction between these two compounds disappears *in vivo* where their cancer chemopreventive efficacies were found to be very similar. The reason for this is that the β-lyase enzyme is abundant in many tissues and thus the animal has ample capacity to convert SeMCYS to methylselenol.

Further work with these mammary cells using methylseleninic acid produced similar results, providing additional support that monomethylated forms of Se are the critical effector molecules in Se-mediated growth inhibition *in vitro* (Sinha *et al.* 1999*a*). Further research is needed to identify why a monomethylated form of Se is required whereas other forms of Se do not have this effect.

Mechanisms of cancer reduction by selenium

A number of reviews have been written on the chemopreventive effects of Se, including most recently those by Combs & Gray (1998), Ip (1998), Ganther (1999), Schrauzer (2000), El-Bayoumy (2001) and Fleming *et al.* (2001). An entire volume of Nutrition and Cancer was devoted to Se and cancer in honour of the late Larry Clark (Cohen, 2001). The mechanism for Se as an anti-carcinogenic element is not known, but several speculative hypotheses have been advanced. It is well established that the most effective dose of Se for cancer protection is at elevated levels, often called supra-nutritional or pharmacological levels. The suggested mechanisms for cancer prevention by Se include its effects upon programmed cell death, effects upon DNA repair, its role in selenoenzymes, its effects upon carcinogen metabolism, its effects upon the immune system, Se as an anti-angiogenic agent and its specific inhibition of tumour cell growth by certain Se metabolites. Detailed discussions have been devoted to the role Se in selenoenzymes, effects on carcinogen metabolism, effects on the immune system, specific inhibition of tumour cell growth and apoptosis (Combs & Gray, 1998), and thus these will be discussed only briefly here.

Role of selenoenzymes

Since GPX act to convert peroxides to less harmful compounds and because peroxidative damage is associated with cancer, it was reasonable to assume that these peroxidases would be involved in the reduction of tumours. However, there is little information to support this possibility. The greatest protection of Se against tumours is at high intakes, but the activities of GPX reach a plateau at nutritional levels with no further increase at higher levels in most tissues. Interestingly, protection by Se as selenite against skin tumours induced in rats either by u.v.-B light (Pence *et al.* 1994) or phorbol esters (Perchellet *et al.* 1987) correlated with the activity of GPX in skin. The hypothesis was advanced that thioredoxin reductase may be involved in reduction of tumours (Ganther, 1999), but experimental results did not support this possibility (Ganther & Ip, 2001). Thioredoxin reductase activity was not affected by high dietary levels of SeMCYS or methylseleninic acid, precursors of methylselenol, in rat liver.

The findings that anti-tumourigenic amounts of Se (≥1·5 mg/kg) reduced tissue lipid peroxidation potential only slightly (Lane & Medina, 1985) or not at all (Horvath & Ip, 1983) suggest that those effects are independent of the function of the GPX. Therefore, at present it is probable that anti-tumourigenic effects of high levels of Se involve mechanisms unrelated to the activities of GPX. The 15 kDa (sep 15) selenoprotein has been suggested as being involved in the reduction of tumours. The sep 15 selenoprotein is localized on chromosome 1p31, a genetic locus commonly mutated or deleted in human cancers (Kumaraswamy *et al.* 2000). The sep 15 selenoprotein genes are manifested at highest levels in prostate, liver, kidney, testis and brain in human subjects and mice; these levels of this selenoprotein are reduced substantially

in malignant prostate cell line and in hepatocarcinoma. Since there is loss of heterozygosity at the sep 15 locus in certain human tumour types, it was suggested that this selenoprotein may be involved in either cancer development or risk, or in both (Kumaraswamy *et al.* 2000).

It is interesting to note that a 15 kDa protein was found in the prostatic epithelium, where it accounted for about two-thirds of the protein-bound [75]Se (Behne *et al.* 1997). Unless the levels of sep 15 can be shown to be elevated with high intakes of Se, the likelihood of its significant involvement in tumour reduction does not appear likely. However, it could still be involved in tumour reduction with nutritional intakes of Se because the tumour suppressor gene and p53 were altered in mice where the selenocysteine tRNA (Ser Sec) gene was deleted in transgenic mice carrying the Cre recombinase gene. This recombinase gene is under control of the mouse tumour virus, suggesting greater susceptibility of these mice to cancer (Kumaraswamy *et al.* 2003).

Effects on carcinogen metabolism

Studies of carcinogen metabolism have yielded varying results. One study showed that comparable dietary levels of Se reduced the formation of covalent DNA adducts of aflatoxin in the chick (Chen *et al.* 1982*b*), but increased this process in the rat (Chen *et al.* 1982*a*). In rats, treatment with Se increased the hydroxylation and subsequent oxidation of azoxymethane (Fiala *et al.* 1991) and to reduce dimethylbenz(a)anthracene–DNA adduct formation (Liu *et al.* 1991), thus reducing the effect of these carcinogens. Se supplementation of rats was shown to reduce the hepatic microsomal production of mutagenic metabolites of several carcinogens, including *N,N*-dimethylaniline (Olsson *et al.* 1984), dimethylbenz(a)anthracene (Martin & Schillaci, 1984), 2-acetylaminofluorene (Chow & Gairola, 1984) and benzo(a)pyrene (Teel & Kain, 1984). These publications indicate that while the effect may not be universal with regard to either carcinogen or host species, high-level Se supplementation can affect carcinogen metabolism by methods that would be expected to inhibit the initiation stage of carcinogenesis.

Effects upon immunity

Since the immunity of cancer patients is reduced and Se has been shown to boost the immune system, it is logical to conclude that Se could reduce tumours by this method. Several studies found that supra-nutritional levels of Se will stimulate the cytotoxic activities of natural killer cells (Koller *et al.* 1986; Peatrie *et al.* 1989; Kiremidjian-Schumacher *et al.* 1996) and lymphokine-activated killer cells (Roy *et al.* 1994). In human subjects, two intervention studies with the same level of Se intake (200 μg/d) shown to reduce cancer risks improved immunity (Kiremidjian-Schumacher *et al.* 1994; Taylor, 1995). The enhancement by Se of the expression of the high-affinity interleukin 2 receptor resulted in an increased capacity to produce cytotoxic lymphocytes and macrophages that can destroy tumour cells (Kiremidjian-Schumacher *et al.* 1996). Up-regulation of the receptor is expected to enhance the clonal expansion of cytotoxic effector cells and thereby modulate T-cell mediated responses in response to signals generated by interleukin 2. Other roles of Se in the immune system are suggested by recent findings that the mRNA of several T-cell-associated genes have open reading frames resembling that of selenoprotein P and potential stem-loop RNA structures with consensus selenocysteine-insertion sequences (Taylor, 1995), suggesting the possibility that they may encode functional selenoproteins yet to be identified. Accordingly, because plasma Se levels, glutathione concentrations and GPX activity are subnormal in HIV-infected individuals (Diamond *et al.* 2001), Se studies were conducted to investigate any relationships. Using ^{75}Se-labelled human Jurkat T cells it was shown that the levels of four ^{75}Se-containing proteins (57, 26, 21 and 15 kDa species) are lower in HIV-infected cell populations than in uninfected cells (Gladyshev *et al.* 1999). SDS–PAGE gels indicated that these Se-containing proteins are subunits of thioredoxin reductase, cellular GPX, phospholipid hydroperoxide GPX and the 15 kDa selenoprotein. There appeared to be greater levels of low-molecular-mass ^{75}Se-compounds in HIV-infected cells than in normal cells. While these results are intriguing, further research is needed on the relationships of selenoproteins to HIV.

Anti-tumourigenic selenium metabolites

It is possible that Se can lead to the formation of selenotrisulfides involving protein sulfhydryl groups that could inhibit sulfhydryl-sensitive enzymes to impair tumour cells metabolism. Se was shown to inhibit bovine pancreatic ribonuclease by forming an intramolecular selenotrisulfide bridge in place of the normal one (Ganther & Corcoran, 1969), and the formation of selenotrisulfides involving the sulfhydryl groups of chick hepatic fatty acid synthase resulted in inhibition of that enzyme activity (Donaldson, 1977). The selenotrisulfide produced by the thiol-dependent reaction of selenite (selenodiglutathione; GS-Se-SG) can be active in inhibiting protein synthesis and enhancing apoptosis (Harrison *et al.* 1996; Pence *et al.* 1996). It should be pointed out, however, that these selenotrisulfides are rather short-lived and somewhat unstable, raising some questions of their long-term effects.

As noted elsewhere, the anti-tumourigenic effects of Se are mediated by the methylated metabolite, methylselenol. Because it inhibits the methylation of selenide, As greatly reduced the anti-tumourigenic effects of selenite, while it enhanced the efficacy of several synthetic Se compounds that are metabolized to methylselenol (Ip & Ganther, 1990, 1992*b*). Several synthetic alkyl and aryl selenocyanates have been evaluated in animal models. The more effective of these are benzylselenocyanate and 1,4-phenylene-bis(methylene) selenocyanate (El-Bayoumy, 1985; Nayini *et al.* 1989). In comparisons with other Se compounds 1,4-phenylene-bis(methylene) selenocyanate (p-XSC) was shown to be more effective against tumourigenesis, but less effective as a source of Se in supporting the expression of GPX and relatively less toxic (Ip & Ganther, 1993). This further suggests that GPX do not play a significant role in counteraction of tumours. Another synthetic Se compound, triphenylselenonium chloride, has

also been found to be anti-tumourigenic (Lu *et al.* 1995), but had only minimum effects in the induction GPX activity. In mice, triphenylselenonium chloride has the greatest safety margin yet observed for any chemopreventive seleno-compounds. The chemopreventive effects of such synthetic seleno-compounds as benzylselenocyanate, p-XSC and triphenylselenonium chloride, which release their Se only very slowly to the general metabolism of the element, may involve more direct effects, perhaps as effective analogues of the anti-carcinogenic metabolites of natural forms of the element (Combs & Gray, 1998).

Selenium and apoptosis

The evidence indicates that one possible mechanism by which Se reduces the incidence of tumours is through its effects upon apoptosis (Sinha *et al.* 1999*a*; Ip & Dong, 2001; Wang *et al.* 2001). Methylseleninic acid produced a more robust response at one-tenth the concentration of SeMCYS on the inhibition of cell proliferation and the induction of apoptosis in mouse mammary epithelial cells (Ip *et al.* 2000*b*). Work with mouse mammary epithelial tumour cells indicates that SeMCYS mediates apoptosis by activating one or more caspases (Unni *et al.* 2001). Of the caspases, caspase-3 activity appeared to be activated to the greatest extent. These cells have ample lyases to convert SeMCYS to methylselenol.

There are some other factors that should be considered concerning Se and apoptosis. The feeding of high levels of dietary Se as selenite to rats increased hepatic concentrations of both reduced glutathione and oxidized glutathione (GSSG) with a decreased reduced glutathione:GSSG ratio (Le Boeuf & Hoekstra, 1983). Similar changes were seen in cultured hepatoma cells treated with high levels of selenite, and Se treatment was found to retard cell-doubling time, increasing the duration of various phases of the cell cycle. Se-induced increases in GSSG may affect protein synthesis, because this oxidized form is known to activate a protein kinase that inactivates eukaryotic initiation factor 2 through phosphorylation (Jacobs *et al.* 1977). It is also inactivated by selenite (Safer *et al.* 1980) or its derivative GS-Se-SG (Vernie *et al.* 1981). GS-Se-SG was found to be more effective in inhibiting the growth of Ehrlich ascites tumours in mice than either the inorganic or amino acid forms of Se (Poirier & Milner, 1983). Apoptotic responses have been demonstrated for cells treated with high levels of selenite (Lu *et al.* 1995), GS-Se-SG (Lanfear *et al.* 1994), p-XSC (El-Bayoumy *et al.* 1992) or triphenylselenonium chloride (Lu *et al.* 1995).

The influence of seleno-compounds upon transcription factor-DNA binding has been summarized by Youn *et al.* (2001). The influence of p-XSC on the binding activities of the transcription factors nuclear factor-κB, activator protein-1, SP-1 and SP-3 were evaluated both *in vitro* and *in vivo*. p-XSC and selenite reduced the consensus site-binding activity of nuclear factor-κB in a concentration-dependent manner when nuclear extracts from cells (HCT-116, a human colo-rectal adenocarcinoma) stimulated with tumour necrosis factor α were incubated with either seleno-compound. However, only p-XSC inhibited nuclear factor-κB consensus recognition site-binding when the

cells were pre-treated with either compound and were then stimulated with tumour necrosis factor α. In contrast, the consensus site-binding activity of activator protein-1 was inhibited with selenite, but not with p-XSC *in vitro* or *in vivo*. p-XSC or selenite reduced the consensus site-binding of transcription factors SP-1 and SP-3 in concentration- and time-dependent manners when nuclear extracts from cells treated with either compound *in vivo* were assayed by electrophoretic mobility shift assay. Interestingly, the S analogue of p-XSC, which is inactive in chemoprevention, had no effect on the oligonucleotide binding of SP-1 and SP-3. Certain genes involved in the inhibition of apoptosis also contain SP-1 binding-sites in their promoter regions (Dong *et al.* 1999). Therefore, it is likely that SP-1 plays an important role not only in the regulation of cell growth and proliferation, but also in programmed cell death. GS-Se-SG will increase the induction and translocation of NF-κB, but decreases its binding to DNA (Galter *et al.* 1994). Although these findings show that very high levels of Se can impair cellular proliferation by enhancing programmed cell death, it is not clear whether they can be extrapolated to living systems in which tissue Se levels tend to be several orders of magnitude less.

The regulation of protein kinase C by Se may be involved in cancer prevention. Protein kinase C is a receptor for certain tumour promoters (Gopalakrishna & Gundimeda, 2002*a*). Oxidant tumour promoters activate protein kinase C by reacting with zinc-thiolates present within the regulatory domain, but in contrast some seleno-compounds such as methylseleninic acid selectively inactivates protein kinase C (Gopalskrishna & Gundimeda, 2002*b*). Interestingly, thioredoxin reductase reverses Se-induced inactivation of protein kinase C. However, this effect was eliminated when the selenocysteine in thioredoxin reductase was either selectively alkylated or removed by carboxypeptidase treatment (Gopalakrishna & Gundimeda, 2002*a*). Similarly, *Escherichia coli* thioredoxin reductase, which is not a selenoprotein, was also not effective, indicating a specific effect of the selenoenzyme. Other studies indicate that the protein kinase C pathway is involved in induction of selenoproteins, thioredoxin reductase and GPX (Jornot & Junod, 1997; Kumar & Holmgren, 1999), further suggesting the influence of this pathway on selenoenzymes.

The induction of apoptosis has been attributed to changes in genes such as cyclin-dependent kinase 2 (cdk2) and gadd45 (Kaeck *et al.* 1997; Sinha *et al.* 1999*b*). The cdk2 and DNA damage-inducible gadd genes are related to cell cycle arrest. *In vitro*, SeMCYS has been reported to arrest mouse mammary tumour epithelial cells at a phase that coincided with a specific block of cdk2 kinase and an elevated expression of gadd34, gadd45 and gadd153 (Kaeck *et al.* 1997). The alterations in cdk2 and gadd45 suggest that the effect of Se in these cells may be related to the P53-mediated apoptosis. The P53 protein is a factor that enhances transcription of several genes, including gadd45.

In general, there is a correlation between the effectiveness of seleno-compounds as chemopreventive agents *in vivo* and their ability to inhibit cell growth and induce apoptosis *in vitro* (Ghose *et al.* 2001). The influence of

GS-Se-SG and p-XSC on normal human oral mucosa cells and human oral squamous carcinoma cells were investigated. Squamous carcinoma cells were significantly more sensitive to induction to apoptosis by GS-Se-SG than normal human oral mucosa cells, but the differences were marginal with p-XSC. Both seleno-compounds induced the expression of Fas ligand in oral cells to a degree that correlated with the extent of apoptosis induction. In addition, both seleno-compounds induced the stress pathway kinases, Jun NH2-terminal kinase and p38 kinases at concentrations causing apoptosis. The human prostate cancer cell line LNCaP exhibited mitochondrial injury and cell death, mainly apoptosis, after acute exposure to selenite (Zhong & Oberley, 2001). Up-regulation by selenite of the cyclin-dependent kinase inhibitor p21 correlated with cell growth inhibitions.

Selenium and DNA repair

It was shown that Semet can activate p53 by a redox mechanism independent of DNA damage (Seo *et al.* 2002*a*). By using a peptide containing only p53 cysteine residues 275 and 277 it was demonstrated the importance of these residues in the Semet-induced response. Mouse embryo fibroblasts wild-type or null for p53 genes was used to obtain evidence that the DNA repair branch of the p53 pathway was activated. In further work, Semet was shown to induce a DNA repair response in normal human fibroblasts *in vitro* and protects cells from DNA damage (Seo *et al.* 2002*b*). It has been estimated that each cell sustains approximately 10 000 potentially mutagenic lesions per d due to endogenous DNA damage and the potential of Se-inducing DNA repair hold great value. Since SeMCYS has been shown to be the most effective seleno-compound against mammary tumourigenesis, it will be interesting to determine if this compound is more effective than Semet in activation of the p53 tumour suppressor protein and thus DNA repair. Work by other researchers indicated that thioredoxin reductase was induced, but GPX was repressed, in malignancies in transgenic mice and prostate cell lines relative to controls (Diamond *et al.* 2001). In the colon cell line, p53 expression resulted in elevated GPX, but repressed thioredoxin reductase. The results indicated that thioredoxin reductase and GPX are regulated in a contrasting manner in the cancer systems tested and reveal the p53 dependent regulation of selenoprotein expression. If Se activates p53 as indicated earlier (Seo *et al.* 2002*a*), then this could be a mechanism whereby Se induces apoptosis because p53 is involved in this programmed cell death. Thus, further investigations into the involvement of Se in DNA repair would appear to be an extremely fruitful avenue to pursue.

Selenium as an anti-angiogenic agent

Angiogenesis, which is the process of formation of new microvessels from existing vessels, is a critical and obligatory component of promotion, progression and metastasis of solid cancers. The chemopreventive effect of increased Se intake against chemically induced mammary carcinogenesis is associated with reduced intra-tumoural

microvessel density and an inhibition of the expression of vascular endothelial growth factor (Lu & Jiang, 2001). The results suggest a methylselenol-specific inhibition of the angiogenic switch mechanism through multiple processes. The evidence indicates that Se exerts its cancer chemopreventive activity through an anti-angiogenic mechanism (Lu, 2000). Mammary carcinomas in Se-fed rats were 24 to 34 % lower than in those animals fed the control diet. The rats had been fed diets with either Se-enriched garlic or selenite. The microvessels in the mammary gland were visualized with immunohistochemical staining and the microvessel number counted. The reduction of small vessels by Se treatment indicated that mechanisms governing the genesis of new vessels was inhibited by this element. Based on data from several laboratories, it was concluded that seleno-compounds that feed into the H_2Se pool will be less desirable as chemopreventive agents for human subjects and conversely, those that enter the methylselenol pool would be more desirable Se forms for human application (Lu, 2000).

Forms of selenium in foods and supplements

The efficacy of various seleno-compounds using the mammary-tumour model is summarized in Table 1. The incidence of breast cancer is greatest for all cancers in women, but it is the third highest cause of all cancer deaths in the USA (American Cancer Society, 2000), probably reflecting the improved methods for the detection and treatment of breast cancer compared with other cancers. Although usually not mentioned, a small number of men develop breast cancer, with even some deaths. About 400 men die of breast cancer per year compared with 43 300 breast-cancer deaths per year in women in the USA.

SeMCYS and selenobetaine are the most effective seleno-compounds identified thus far against mammary tumourigenesis in animals (Table 1). Although selenobetaine is just as effective, SeMCYS is considered to be the most interesting seleno-compound, because it is the predominant form present in Se-enriched plants such as garlic (Ip *et al.* 2000*a*), broccoli florets (Cai *et al.* 1995), broccoli sprouts (Finley *et al.* 2001) and onions (Cai *et al.* 1995). Selenobetaine has never been detected in

Table 1. Anti-carcinogenic efficacy of different selenium compounds for the reduction of mammary tumours in rats (from Ip & Ganther, 1993 and Ip *et al.* 1994*a,b*)

Compound	Dietary Se (µg/g) for 50 % inhibition
Se-methylselenocysteine	2
Selenobetaine	2
Selenobetaine methyl ester	2–3
Selenite	3
Selenomethionine	4–5
Selenocystine	4–5
p-XSC	8–10
Triphenylselenonium	10–12
Dimethylselenoxide	>10
Trimethylselenonium	No effect at 80 µg/g

p-XSC, 1,4-phenylene-bis(methylene) selenocyanate.

Se-enriched plants. Therefore, SeMCYS has received the most recent attention as possibly the most useful one for cancer reduction. Except for Semet and selenocystine, the other seleno-compounds listed in Table 1 are not present in plants and thus are mostly of academic interest. However, some of them are of therapeutic interest. Selenobetaine and SeMCYS are good precursors for generating monomethylated Se (Ip, 1998; Ip & Ganther, 1993). Selenobetaine tends to lose a methyl group before scission of the Se–methylene-C bond to form methylselenol. SeMCYS is converted to methylselenol directly when cleaved by β-lyase, and unlike Semet it cannot be incorporated non-specifically into proteins. These seleno-compounds can be converted directly to methylselenol: this is presumably the reason they are more efficacious than other forms of Se.

Dimethylselenoxide and selenobetaine methyl ester are converted to dimethylselenide, but are less effective for reduction of tumours (Ip, 1998). Trimethylselenonium is essentially not effective in tumour reduction. Thus, there is a negative correlation between the effectiveness of these seleno-compounds and the degree of methylation.

Even though Semet is effective against mammary tumours, one disadvantage as noted earlier is that it can be incorporated directly into general proteins instead of converted to compounds that reduce tumours most effectively (Ip, 1998). When this occurs its efficacy for tumour reduction is reduced. For example, when a low-methionine diet was fed, there was significant reduction in the protective effect of Semet even though the tissue Se was actually higher in animals as compared with those given an adequate amount of methionine (Ip, 1988). When methionine is limiting, a greater percentage of Semet is incorporated non-specifically into body proteins in place of methionine, because the methionine-tRNA cannot distinguish between methionine and Semet. Feeding diets with Semet to animals as the main Se source will result in greater tissue accumulation of Se than other forms of Se (Whanger & Butler, 1988; Ip & Lisk, 1994). It is not known whether this stored Se can serve as a reserved pool of this element, but the evidence indicates that it is metabolically active (Waschulewski & Sunde, 1988).

With the knowledge of the effects of these seleno-compounds as anti-carcinogenic agents, it was of interest to investigate the most appropriate methods for delivery to the general population. One obvious approach was to investigate additional methods for expeditious ways to deliver these protective agents through the food system. One strategy in this direction was the investigation of enriching garlic with Se (Ip et al. 1992). The addition of Se-enriched garlic to yield 3 μg Se/g diet significantly reduced the mammary-tumour incidence in rats from 83 to 33 %. Similarly to garlic, Se-enriched broccoli also reduced mammary-tumour incidence from 90 to 37 % (Finley et al. 2001).

Se-enriched garlic was shown to be twice as effective as Se-enriched yeast in the reduction of mammary tumours (Table 2). The total number of tumours as well as the incidence of tumours was reduced to a greater extent by enriched garlic than enriched yeast. Chemical speciation of Se in these two products indicated that Semet was the predominant form of Se in enriched yeast, whereas SeMCYS (as the glutamyl derivative) was the predominant form of Se in enriched garlic (Ip et al. 2000a). The glutamyl derivative is considered a carrier of SeMCYS and both of these compounds were shown to be equally effective in the reduction of mammary tumours (Dong et al. 2001). These results are consistent with those in Table 1, where SeMCYS was more effective than Semet for reduction of mammary tumours. The chemical composition of seleno-compounds in these two sources of Se is apparently responsible for this difference in efficacy. However, it is not known whether doubling the amount of Se as Se-enriched yeast will be as effective as enriched garlic. Neither is it known whether the combination of enriched yeast and enriched garlic would be more effective than either alone.

Using another model, Se-enriched broccoli florets (Finley & Davis, 2001; Finley et al. 2001, 2002) as well as enriched broccoli sprouts (Finley et al. 2001) significantly reduced colon tumours in rats. This is intriguing because colon cancer is the third most common newly diagnosed cancer in the USA, resulting in about 55 000 deaths per year due to this type of cancer (American Cancer Society, 2000). Se-enriched broccoli was more effective than selenite, selenate or Semet in the reduction of induced colon carcinogenesis (Davis et al. 1999; Feng et al. 1999; Finley & Davis, 2001). In contrast, selenite, selenate and Semet were more effective for induction of GPX activity than Se-enriched broccoli (Finley et al. 2000). This indicates that the plant converts the Se to more effective forms for reduction of these tumours and these results emphasize the need to study the effects of Se in food forms.

Similar to chemically induced colon tumours, there were significantly fewer intestinal tumours when mice that have

Table 2. Mammary cancer prevention by selenium-enriched garlic or selenium-enriched yeast in the dimethyl(a)anthracene (DMBA) and methylnitrosurea (MNU) models (from Ip et al. 2000)

Model	Treatment	Dietary Se (μg/g)	Tumour incidence	Total no. of tumours (n)	Inhibition (%)‡
DMBA	None	0·1	26/30	74	
	Se-enriched garlic	3·0	11/30*	25*	66
	Se-enriched yeast	3·0	19/30†	49†	34
MNU	None	0·1	28/30	80	
	Se-enriched garlic	3·0	10/30*	24*	70
	Se-enriched yeast	3·0	20/30†	55†	31

Mean values were significantly different from those of the corresponding Se-enriched yeast group: *$P < 0.05$.
Mean values were significantly different from those of the corresponding control group: †$P < 0.05$.
‡ Calculated based on total tumour yield data.

a genetic defect for development of intestinal tumours were fed Se-enriched broccoli (Davis *et al.* 2002). These results, along with previous data, indicate that Se-enriched broccoli is effective against both chemically and genetically induced intestinal tumours. Data from work with another strain of mice that develop spontaneous intestinal tumours is consistent with these results where Se deficiency resulted in activation of genes involved in DNA damage (Rao *et al.* 2001).

Level of selenium necessary for nutritive benefit

The Chinese results have been used almost exclusively to establish the required levels of Se for nutritive benefit as well as to establish the safe levels for protecting human health (Yang *et al.* 1989*b*; Yang & Zhou, 1994). It is fortunate to have a country like China where areas vary from deficient to toxic levels of Se, and this has made it convenient to collect critical information on the metabolism and effects of various levels of Se in human subjects. Significant correlations have been found between daily Se intake and Se content of whole blood, plasma, breast milk and 24 h urine (Yang *et al.* 1989*a*). Highly significant correlations were also found between levels of whole-blood Se and hair Se, fingernail Se and toenail Se, hair Se and fingernail or toenail Se, and whole-blood Se and toenail or fingernail Se. Morphological changes in fingernails were used as the main criteria for clinical diagnosis of selenosis (Yang *et al.* 1989*b*). The fingernail changes and loss of hair are the main signs of excess Se intakes. With excess Se intakes, the fingernails become brittle and are easily cracked. The data collected on Chinese subjects are summarized in Table 3.

An intake of nearly 5 mg Se/d resulted in definite occurrence of selenosis, characterized by hair and nail losses. It has been suggested that the subjects were able to tolerate this high level of Se because they consumed a high-fibre diet. The low adverse effect level of dietary Se was calculated to be 1540–1600 μg/d. However, some effects were noted in individuals with an intake of 900 μg/d. The maximum safe dietary Se intake was calculated to be about 800 μg/d, but there were some individuals where an amount of 600 μg/d was the maximum safe intake. In order to provide a safety factor, the maximum safe dietary Se intake was suggested as 400 μg/d. A level of about 40 μg/d was suggested as the minimum requirement, while an intake of <11 μg/d will definitely result in deficiency problems. Deficiency of Se in humans results in a cardiac and muscular disorder called Keshan disease, and deficiency of Se is thought to be one of the contributing factors to a joint disorder called Kashin–Beck disease.

Conclusions and future research

Doses of 100–200 μg Se/d inhibit genetic damage and cancer development in human subjects. About 400 μg Se/d is considered an upper safe limit. The recommended daily allowance for Se is 55 μg/d for both men and women (Food and Nutrition Board, Institute of Medicine, 2000); the FAO/WHO has set 26 and 34 μg/d for women and men respectively (Food and Agriculture Organization/World Health Organization, 2002). Clearly, doses greater than the recommended daily allowance or FAO/WHO levels are needed to inhibit genetic damage and prevent cancer. Despite concerns about the toxicity of higher dietary levels of Se, human subjects consuming up to 600 μg/d appear to have no adverse clinical symptoms. The author is aware of a person who consumed 1 mg Se (as selenite) for 2 years before toxic signs of Se occurred and these disappeared when Se consumption was stopped. Thus, this element appears not to be as toxic as is often believed.

Table 3. Health effects of various levels of dietary selenium intakes in China (modified from Yang & Zhou, 1994)*

Average adult dietary Se intakes		Forms	Effects on human health
μg/d	μg/kg body weight		
4990 (SD 1349)†	90	Cereal-based plant diet in seleniferous area	Occurrence of selenosis with hair and nail loss
1660†	30	Cereal-based plant diet in seleniferous area	AEL of dietary Se intake
1540 (SD 653)†	28	Cereal-based plant diet in seleniferous area	Mean LOAEL of dietary Se intake
0·900†	17	Cereal-based plant diet in seleniferous area	Individual low level causes toxicity (individual LOAEL)
819 (SD 129)†	15	Cereal-based plant diet in seleniferous area	Maximum safe dietary Se intake (mean NOAEL)
600†	11	Cereal-based plant diet in seleniferous area	Individual maximum safe dietary Se intake (individual NOAEL)
400	–	Natural diet	Suggested maximum safe dietary Se intake
40	0·7	75 % Dietary Se from selenomethionine	Suggested adequate dietary Se requirement
<11	<0·2	Cereal-based plant diet in Keshan disease area	Prevalence of Keshan disease and Kaschin–Beck disease

AEL, adverse effect level; LOAEL, low adverse effect level; NOAEL, no adverse effect level.
* Calculated by regression equation.
† Data modified from those of Yang & Zhou (1994).

Information from both animal and human research indicate that > 100 and up to 200 μg additional Se/d are necessary for greatest reduction of cancer. This is because a methylated form of Se is necessary for maximum reduction of mammary cancer, and this methylated form is present at highest levels with elevated intakes of this element. In most human trials, the subjects were supplemented with 200 μg/d and in trials where only 50 μg/d was given, the reduction in the incidence of cancer was not as great. Therefore, it is concluded that the Se requirement for maximum reduction of cancer appears to be at least four times the recommended daily allowance. However, since only 50–200 μg additional Se/d have been used, it is not possible to indicate which level will give maximum protection. For example, it is not known whether supplemental levels of Se > 200 μg/d in addition to the dietary intake of Se will provide any additional protection against cancer.

In the mammary-tumour model, evidence indicates that methylselenol is the active form of Se against tumour formation. Whether this is true in other tumour models, such as the colon, remains to be determined. It is not known why methylselenol is effective in mammary tumour reduction, whereas other forms of Se do not have this effect. Therefore, future research should be focused on the mechanism of mammary tumour reduction by methylselenol, and this should provide information on why other forms of Se cannot serve this function. Because methylselenol is volatile, the use of precursors of this compound appears to be a fruitful avenue to pursue. SeMCYS is the most effective seleno-compound found in enriched plants for conversion to methylselenol. It is speculated that the reason this seleno-compound is more effective in mammary tumour reduction is because it can be converted directly to methylselenol, whereas other seleno-compounds found in enriched plants must be converted to this methylated form through several metabolic steps. In contrast to mammary tumours, preliminary results indicate that SeMCYS may not be the most effective seleno-compound against colon tumours, suggesting that another seleno-compound (or seleno-compounds) is the most effective one against tumours in this tissue (PD Whanger, unpublished results).

There are several hypotheses on the mechanism by which Se reduces tumour formation. The most likely ones appear to be increased apoptosis, increases in DNA repair, and Se acting as an anti-angiogenic agent or possibly through a selenoprotein. Since the data for all four of these possibilities appear sound, it is proposed that Se does not reduce tumourigenesis by a single mechanism, but instead by multiple ones. Future research should be concentrated on which of these are the most important and how to improve the efficacy of methylselenol or other seleno-compounds as in the case of colon tumours. For example, molecular biologists should use genetic engineering to increase the content of SeMCYS or other effective seleno-compounds in plants such as garlic, broccoli and onions for maximum benefits of Se-enriched plants. There is evidence that the pure compound may give different results as compared with its presence in the plant. For example, Semet is not very effective in reduction of colon tumours, but Se-enriched wheat, where the major form of Se is Semet, is highly effective in the reduction of tumours in this tissue (Finley & Davis, 2001). Similar results have been found with Se-enriched broccoli where the major form of Se is SeMCYS. Enriched broccoli is very effective in the reduction of colon tumours, whereas pure SeMCYS does not appear to be as effective. Another possibility is that the seleno-compounds are interacting with other components in the plant to produce more effective results than the pure compound alone. This possibility would appear to be a fruitful avenue to pursue.

Se-enriched yeast is the most common source of Se available commercially and it also has been the most used Se source in trials with human subjects. Semet is the major form in enriched yeast, but SeMCYS is the predominant form in enriched plants such as garlic and broccoli. Se-enriched garlic was shown to be twice as effective as enriched yeast in reduction of mammary tumours in rats. However, it is not known whether providing twice as much Se as enriched yeast will give the same benefits as enriched garlic. Therefore, in addition to enriched yeast, Se-enriched food plants such garlic and broccoli should be also an effective and safe method for delivery of Se to the general population. Future research should involve the use of a combination of enriched yeast and enriched vegetables such as broccoli or garlic to determine whether there is a synergistic effect in tumour reduction. Nevertheless, regardless of the source of Se it is apparent that additional intakes of this element by human subjects will reduce the incidence of cancer. It has been estimated that one-third of the cancers in humans are environmentally related. The results in the present report indicate that on an average there could be 50 % reduction of cancer through increased Se ingestion in human subjects. If the 50 000 deaths due to colo-rectal cancer, 41 800 deaths due to prostate cancer in men or 43 300 breast cancer deaths per year in US women could be reduced by one-half with Se, this would be a very significant contribution to human health.

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