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Metabolic and Functional Defects in Selenium Deficiency [and Discussion]

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Metabolic and functional defects in selenium deficiency

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This paper is concerned with present-day knowledge of the biological role of selenium, of its interaction with other nutrients including trace elements, and with the importance of selenium in human nutrition and health.

Selenium has been shown to be an integral part of glutathione peroxidase, which catalyses the reduction of a large range of lipid hydroperoxides and hydrogen peroxide. The interrelation between vitamin E, selenium and polyunsaturated fatty acids is complex. First, selenium in glutathione peroxidase may control intracellular levels of hydrogen peroxide, which affect the formation of active oxygen metabolites that may serve as initiators of lipid peroxidation; this role of selenium is closely related to that of superoxide dismutases, which control intracellular levels of the superoxide anion. Secondly, vitamin E may control the formation of lipid hydroperoxides through its antioxidant function, as well as possibly entering into a structural relation with membrane phospholipids. Thirdly, glutathione peroxidase may catalyse the reduction of lipid hydroperoxides, formed from membrane lipids, to hydroxyacids without detriment to the cellular economy.

In the field of human nutrition, the lack of selenium has been shown to be the cause of a cardiomyopathy known as Keshan disease, occurring in the People's Republic of China. Blood selenium levels in patients from this area are compared with blood selenium levels in three other parts of the world and the conclusion is reached that the blood selenium level of populations in Keshan disease regions are exceptionally low and that Keshan disease is the first demonstration that selenium is an essential trace element for man.

1. Introduction

Selenium, in common with several other trace elements, is of particular interest because it is both toxic, at high levels of intake, and essential, at lower levels of intake, to living creatures. The toxicity of selenium has been known since the early 1930s (Robinson 1933; Franke 1934) when studies of the disorders that had been observed among cattle and horses in South Dakota, U.S.A., revealed that these were associated with the ingestion of wheat or forages containing selenium at up to 12 μg/g. The discovery of the essentiality of selenium stems from early observations of Schwarz (1944) that rats given a diet low in vitamin E and sulphur-containing amino acids died from a necrotic degeneration of the liver. Subsequent work by Schwarz and his coworkers led to the identification of selenium as the antinecrogenic factor (Schwarz *et al.* 1957). It was, however, further shown that either vitamin E or selenium could prevent liver necrosis and that the dose of selenium required was about one five-hundredth of the dose of vitamin E required to give complete protection from the disease. Furthermore, these nutritional requirements were increased when the level of dietary polyunsaturated fatty acid was increased, demonstrating clearly the close nutritional relationship that exists between the three

nutrients. Research in the intervening years has been directed towards providing an explanation of this interaction. This paper is concerned with reviewing current knowledge of the biological role of selenium and of its interaction with other nutrients and trace elements, and with the importance of selenium in animal and human nutrition and health.

2. Biochemistry of selenium in eukaryotic organisms

The breakthrough in our understanding of the metabolic role of selenium came in 1973 with the discovery in Hoekstra's laboratory at Madison, Wisconsin, U.S.A., that selenium is intimately involved with the enzyme glutathione peroxidase (Rotruck *et al.* 1973). Before then, circumstantial evidence had led to the conclusion that the biological role of vitamin E and selenium was involved in some way with peroxides. Since 1973, the truth of this conclusion has become apparent and the role of these nutrients is clearly seen as being central to the control of oxygen metabolites in eukaryotic organisms. In prokaryotes, such a role has not been demonstrated, although selenium has been shown to be involved in a number of bacterial enzymes that are not found in eukaryotes (Stadtman 1974). An understanding of the role of selenium and vitamin E in oxygen metabolism in eukaryotic organisms requires a brief review of this topic, although it is dealt with in more detail by Hill (this symposium).

It is now generally agreed that, in addition to the reduction of oxygen in biological electron transfer processes, oxygen is also reduced in biological systems by a monovalent process involving the sequential addition of single electrons resulting in the formation of products, such as the superoxide anion, O_2^-, that are potentially damaging to living cells. The monovalent reduction of dioxygen can be summarized as follows:

$$O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-} O_2^{2-};$$

$$O_2^{2-} \xrightarrow{H^+} HO_2^- \xrightarrow{H^+} H_2O_2;$$

$$H_2O_2 \xrightarrow{e^-} OH^- + OH^{\cdot};$$

$$OH^{\cdot} \xrightarrow{e^-} OH^-.$$

A further oxygen derivative that may be of significance in the present context is singlet oxygen ($^1\Delta_g O_2$), which may be formed, for example, by the effect of radiation on dioxygen. In certain eukaryotic cells, however, it may be formed through the mediation of myeloperoxidase, which catalyses the following reactions:

$$O_2 + Cl^- \longrightarrow HOCl^-;$$

$$HOCl^- + O_2 \longrightarrow HOOCl + {}^1\Delta_g O_2.$$

Clearly, many of these oxygen derivatives are highly reactive and their formation *in vivo* could, if uncontrolled, lead to extensive damage to living cells. While there is a natural tendency of the superoxide anion (O_2^-) to disproportionate to hydrogen peroxide and oxygen, and for hydrogen peroxide to disproportionate to water and oxygen, the process is accelerated *in vivo* with O_2^- by superoxide dismutase, and with H_2O_2 by peroxidases and catalase. In the absence of these enzymes, it is likely that further potentially damaging species, such as the hydroxyl radical, might be formed, and in this connection the Haber–Weiss reaction is of particular significance (Haber *et al.* 1934):

$$O_2^- + H_2O_2 \longrightarrow O_2 + OH^- + OH^{\cdot}.$$

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Further, catalysis by a redox-active metal such as iron might be involved *in vivo*, with concomitant formation of other powerful oxidant species such as $[FeO_2]^+$:

$$O_2^- + Fe^{III} \longrightarrow O_2 + Fe^{II}$$
$$H_2O_2 + Fe^{II} \longrightarrow OH^- + OH^{\cdot} + Fe^{III}$$
$$O_2^- + Fe^{II} \longrightarrow [FeO_2]^+$$

Formation of hydroxyl radicals and $[FeO_2]^+$ in significant amounts would therefore have catastrophic consequences for living cells. The necessary function of selenium, in glutathione peroxidase as well as other peroxidases and catalase, is thus in catalysing the destruction of H_2O_2 (and, in glutathione peroxidase, lipid hydroperoxide as well) before potentially deleterious products can be formed from the peroxide. Moreover, the roles of copper, zinc and of manganese and iron in superoxide dismutases of the cytosol and mitochondria are intimately associated with the role of selenium; this topic is considered elsewhere in this symposium by Hill.

The catalytic activity of glutathione peroxidase depends on its containing 4g-atoms of selenium per mole of enzyme. It is an enzyme containing four equal subunits, each of which contains one atom of selenium. The relative molecular mass of the enzyme is about 84000, but this varies somewhat with species (Flohe 1979). Although the specificity of the enzyme with respect to glutathione is very high (even closely related derivatives of glutathione cannot act as substrates), the enzyme will catalyse the reduction of many peroxides, ranging from hydrogen peroxide to various lipid hydroperoxides (Little *et al.* 1968). The complexity of the reaction kinetics of glutathione peroxidase has been reviewed by Flohe (1971); the maximum velocity and limiting Michaelis constants are indeterminate, since they vary with the glutathione concentration over a wide range of values and also with the nature of the primary substrate, be it hydrogen peroxide or a lipid hydroperoxide. Despite some early indications to the contrary, it now seems clear that the selenium is located at the catalytic site of the enzyme and that it participates in the catalytic activity. The selenium is present as selenocysteine (Forstrom *et al.* 1978), which is generally thought to be formed by a post-translational modification of a cysteinyl residue in the apoprotein, perhaps requiring the utilization of selenium in the selenide oxidation state (Diplock *et al.* 1973; Diplock 1976). However, Hawkes *et al.* (1979) have produced some evidence for the existence of a selenocysteinyl-tRNA whose function, they suggest, is to bring about the insertion of a complete selenocysteinyl residue during synthesis of the polypeptide.

The role of glutathione peroxidase in catalysing the removal of lipid hydroperoxides is, in a sense, secondary to the more fundamental role of vitamin E in the control of peroxidation of unsaturated fatty acid residues in membrane phospholipids. The role of vitamin E and of selenium may thus be seen respectively as a first and second line of defence against the damaging effects of active oxygen derivatives. However, the initiation of peroxidation in polyunsaturated fatty acids probably involves the superoxide anion, hydroxyl radicals or singlet oxygen (see, for example, Kellogg *et al.* 1975; Fridovich 1979; Diplock 1981). The control of the initiation of peroxidation thus may depend on glutathione peroxidase, since a factor in the generation of active oxygen species is the intracellular level of hydrogen peroxide. The interrelation of vitamin E, selenium and polyunsaturated fatty acids thus involves a complex interplay of the three factors, which may be seen at three distinct levels. First, selenium in glutathione peroxidase may function in the control of intracellular levels of hydrogen peroxide, which in turn will affect the formation of oxygen metabolites that may serve as initiators of lipid peroxidation.

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This role of selenium is closely related to that of superoxide dismutases in controlling intracellular levels of the superoxide anion. Secondly, vitamin E may control the formation of hydroperoxides in polyunsaturated fatty acyl residues of membrane phospholipids, a process which, in addition to the classical antioxidant role of the vitamin, may involve α-tocopherol entering into a structural relation with membrane phospholipids as was proposed by Diplock & Lucy (1973). Thirdly, glutathione peroxidase may act by catalysing the reduction of lipid hydroperoxides, formed from membrane polyunsaturated fatty acids, to hydroxyacids, which may then be further metabolized without detriment to the cell.

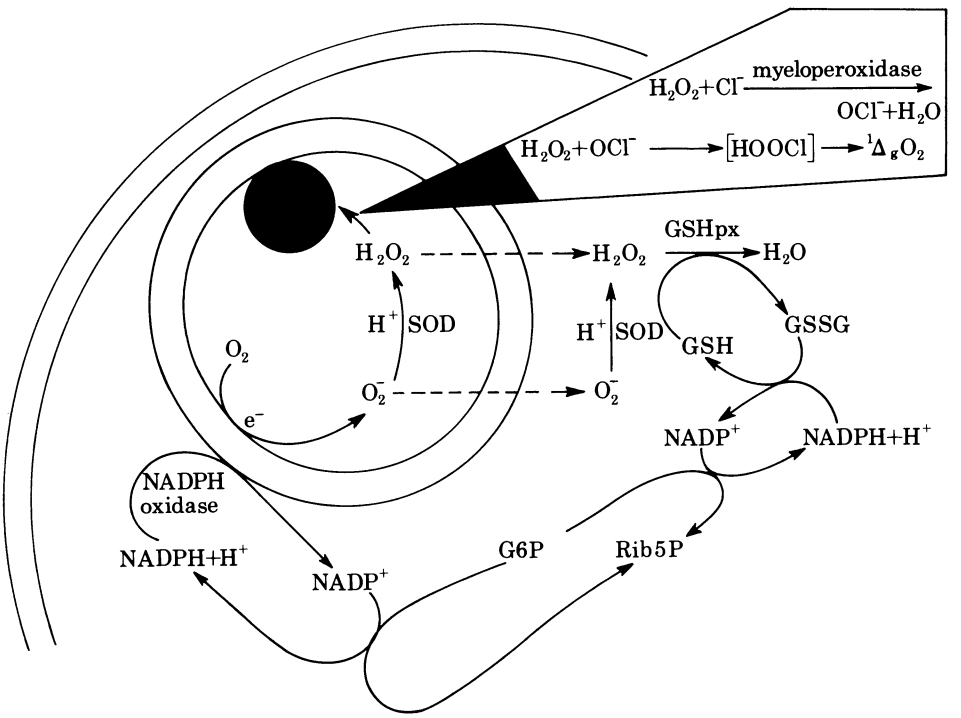

FIGURE 1. Schematic representation of events during oxidative destruction of a bacterium in a leucocyte. The bacterium is represented as the dark circle enclosed within a phagosome formed by invagination and 'pinching off' of the plasma membrane. The oxygen burst is accompanied by the generation of NADPH, which is utilized by NADPH oxidase in the generation of active oxygen derivatives from dioxygen.

Many of the features of the scheme outlined in the preceding paragraph are illustrated by reference to the process of phagocytosis by leucocytes, detailed knowledge of which has emerged recently. After the phagocytic ingestion of bacteria by leucocytes, there is a large increase in oxidative metabolism, known as the 'respiratory burst', that involves a 10–15-fold increase in oxygen consumption, which has been shown to be caused by increased oxidation of glucose via the hexose monophosphate shunt. A major metabolite formed is hydrogen peroxide (Iyer *et al.* 1961), which is produced from the superoxide anion generated in large quantities during the respiratory burst (Babior *et al.* 1973). The hydrogen peroxide is considered to be responsible for killing the bacteria and it is probable that the myeloperoxidase reaction, which gives rise to singlet oxygen (and possibly hydroxyl radicals), is involved (Klebanoff *et al.* 1979). Figure 1, which has been adapted from Roos *et al.* (1979), is a representation of these reactions. The plasma membrane NADPH oxidase (and also possibly NADH oxidase) is likely to be respon-

sible for the generation of the superoxide anion during the 'respiratory burst'. Since the phagosome is formed by invagination of the plasma membrane, the enzyme will be present in the phagosome where, during the 'respiratory burst', large amounts of superoxide will be formed. It is unlikely that the high levels of superoxide anion and hydrogen peroxide so formed will be contained within the phagosome, but some leakage will occur of both metabolites. Cytosolic superoxide dismutase and glutathione peroxidase are available to catalyse their disproportionation, the membranes concerned may be protected by vitamin E, and disastrous consequences to the leucocytes are therefore prevented.

3. Interactions of selenium with other trace elements

(*a*) *Copper, zinc, manganese and iron*

The close relationship of selenium biochemistry with copper, zinc manganese and iron in the superoxide dismutases has already been referred to in the preceding section (see also Hill, this symposium).

(*b*) *Arsenic*

It was shown by Moxon (1938) that the chronic or acute selenoses produced in livestock by feeding seleniferous grains containing selenium at 15 μg/g could be alleviated or prevented by feeding arsenic as $NaAsO_2$ at 5 μg/g. Further work was undertaken by Ganther in Baumann's laboratory at Madison, Wisconsin, and by Levander, who showed that the excretion of selenium via the gastrointestinal tract was enhanced by arsenic (Ganther *et al.* 1962) and that graded doses of arsenic produced a progressive rise in the faecal excretion of a standard dose of selenium and a corresponding fall in the liver selenium level (Levander *et al.* 1966*a*). Subsequently it was shown (Levander *et al.* 1966*b*) that, in rats with acute biliary fistulas, the biliary excretion of selenium was enhanced tenfold by arsenic administration, which indicates that, in addition to the familiar urinary and exhaled air routes of selenium excretion (Diplock 1976), the biliary route may have physiological significance. Levander (1972) has suggested that arsenic forms a detoxication conjugate with selenium, and Parizek (1974) has reported that the enhanced biliary excretion of selenium after arsenic administration is accompanied by a fall in the quantity of dimethyl selenide exhaled. More recently, arsenic was shown to have little effect on tissue glutathione peroxidase levels (Levander *et al.* 1980; Diplock *et al.* 1980) and that, in vitamin E-deficient rats, arsenite caused a significant lowering of the selenium level of several tissues, an effect which was not seen in the tissues of rats supplemented with vitamin E (Diplock *et al.* 1980).

(*c*) *Cadmium and mercury*

The interaction of selenium with cadmium and mercury has been reviewed by Parizek *et al.* (1974) and Diplock (1976). Only the salient features will be given here, since there has been little progress in this field in the intervening years. The toxic effect of both cadmium (given as cadmium salts) and of mercury (given either as mercury salts or or as organic mercurials) is markedly lowered by administration of amounts of selenium that by themselves would be toxic. The nature of the interaction between selenium and these two heavy metals remains obscure, but there is evidence that the interaction of selenium with cadmium differs from the interaction of selenium with mercury. Paradoxically, administration of selenium results, in addition to a lowered toxicity of cadmium and mercury, in a fall in the excretion of both cadmium and mercury, and a corresponding rise in the level of the heavy metals in the blood plasma. It

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therefore seems likely that the effect of selenium in reducing the toxicity of these metals is by forming a complex with them, which, although it is cleared less rapidly from the body, renders the metal less likely to exert its toxic effect on the body tissues. Evidence for this 'complex formation' suggestion has also been obtained from studies of the effect of cadmium and mercury on the excretion of selenium. The metals inhibit the formation of dimethyl selenide, which is a major pulmonary excretory product of selenium, suggesting that the selenium is required elsewhere in forming complexes with cadmium or mercury. The nature of the complexes formed is unknown and there is a need for study of this and other related questions.

(*d*) *Silver*

Silver was shown to be toxic to rats deficient in vitamin E by Mason (Shaver *et al.* 1951; Mason 1953). In a later systematic study with both chicks and rats (Diplock *et al.* 1967; Bunyan *et al.* 1968), it was shown that 0.15% silver acetate in the diet was without effect in rats and chicks when they were given nutritionally adequate amounts of dietary vitamin E and selenium. When vitamin E was withdrawn, the silver produced toxic effects in both species; in rats, centrilobular hepatic necrosis resulted, which was rapidly fatal and which, by light and electron microscopic criteria, was indistinguishable from the liver necrosis caused by selenium and vitamin E deficiency (Grasso *et al.* 1969). In chicks, a pro-exudative effect was demonstrated, similar to the exudative diathesis characteristic of vitamin E and selenium deficiency in this species. It was concluded that, under conditions when vitamin E levels are limiting, silver, by forming a complex with selenium, limits the availability of selenium for glutathione peroxidase formation. The nature of the complex has not been established, but it is noteworthy that there is evidence that in eukaryotes selenium undergoes reduction before being incorporated into glutathione peroxidase (Diplock *et al.* 1973). Silver selenide is extremely insoluble and it seems likely that silver may interfere with the metabolic transformations of selenium by removing selenide as the insoluble silver salt.

4. Selenium in the nutrition of farm livestock and of human populations

Selenium deficiency in farm livestock is a readily identifiable condition that can be readily treated. Among methods available for this are: treatment at source by top-dressing with sodium selenite of crops and forage-yielding pastures known to be growing on soils deficient in selenium, addition of selenium to feedstuffs containing inadequate amounts of the element, and the implantation of selenium 'bullets' in cattle and sheep that graze pastures deficient in selenium.

Of great current interest is the possible incidence of selenium deficiency in man. Attention has been focused on this important problem by the discovery, in the People's Republic of China, of a selenium-responsive cardiomyopathy called Keshan disease. In the following account, information about Keshan disease is summarized and a comparison made with the selenium status of dietetically treated children suffering from phenylketonuria and maple-syrup urine disease in the Federal Republic of Germany. Reference will also be made to a recent experiment by Levander in which human volunteers were subjected to a selenium depletion–repletion regimen.

Although Keshan disease was recognized as early as 1935, it was only after a systematic study of the disease that it was appreciated that it could be prevented by the administration

of sodium selenite. Reports of this important work eventually reached the West, and, while the range of papers describing the detailed work on the disease are not readily accessible because they are written in Chinese, an excellent summary in English is now available (Chen *et al.* 1980).

Keshan disease is an endemic cardiomyopathy characterized by multiple focal myocardial necrosis scattered throughout the heart muscle and exhibiting different degrees of cell infiltration and fibrosis. Clinically, it has been divided into four subtypes: (i) acute, with cardiogenic shock, heart–brain syndrome, pulmonary oedema and severe arrythmia; (ii) chronic, with moderate to severe cardiac enlargement and congestive heart failure; (iii) subacute, intermediate in severity between the proceding types, with facial oedema and gallop rhythm, usually occurring in children; and (iv) latent, with normal heart function and mild cardiac enlargement. In the first three categories the mortality is high, and the patients affected are children of both sexes and women of childbearing age. Incidence of the disease is restricted to certain areas with strongly leached hill and mountain soils and to some 'black earth' soils, and extends in a broad band across China, from the northeast to the southwest. Within the affected areas are numerous 'safe islands' where the disease never occurs, and, because of the extraordinary distribution of disease incidence, it has for many years been regarded as a 'geochemical disease' (see Tang 1979). The similarity of the disease to 'mulberry heart disease' in pigs, the fact that the associated degeneration of muscles of the legs of some Keshan disease patients was similar to white muscle disease in lambs, and the incidence of selenium deficiency myopathies in farm livestock in the affected areas (Hsu, personal communication), led Chinese scientists to suspect that Keshan disease might be caused by selenium deficiency. Trials conducted in the years 1966–9 in which sodium selenite was administered to children in affected areas gave encouraging results. A nationwide programme was therefore instituted in which large groups of children from affected areas were given doses of sodium selenite and compared with children from the same areas given a placebo. The selenium content of 'scalp' hair (i.e. hair close to the head), whole blood and urine were measured, and the selenium content of staple cereal crops grown locally was also determined. A highly significant correlation was found between hair and blood selenium levels over a wide range of selenium contents, and in some of the later studies hair selenium levels only were used as an index of selenium status because collection of hair samples could be carried out among peasant children by unskilled workers. It quickly became apparent that the hair selenium levels of children from affected areas were very low and that selenium administration was lowering the incidence of Keshan disease.

In 1974, the preventive effect of selenium was tested in all children of 1–9 years of age in 119 'production teams' of three communes, and was extended in 1975 to 169 teams of four communes in Sichuan province. Half the children were given sodium selenite tablets orally (0.5–1.0 mg/week) and the remainder were given a placebo. In 1976 and 1977 all children in the communes were given the sodium selenite dosage, since it became apparent that the preventive effect of selenium was so remarkable that it would be unethical to withold selenium from a further control group of children. The results of this study are given in table 1, from which it is apparent that, by the end of 1977, Keshan disease had been completely controlled in these children. Since 1977, treatment with sodium selenite has been extended to all areas in which Keshan disease is endemic, and the disease is now quite rare. Recently, an intensive study of various biochemical aspects has been undertaken, as well as an investigation of the

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clinical aspects of the disease. In all affected areas studied, selenium levels in staple foods and in populations eating them were found to be very low; thus the hair selenium level in unaffected areas was 0.2–0.8 μg/g, whereas in the affected areas it was 0.03–0.12 μg/g. Detail of this work is given by Chen *et al.* (1980), and tables 2 and 3 give some results taken from that paper.

It has thus been amply demonstrated that selenium is involved in the aetiology of Keshan disease, which appears to be uniquely confined to the People's Republic of China (although it may exist in Korea and Japan (Hsu, personal communication)). However, despite the rarity of the disease on a worldwide basis, it can be concluded from these studies that selenium is an

Table 1. Incidence and prognosis of Keshan disease in selenium-treated and control children in Sichuan Province, 1974–7

(Treated children were given a tablet weekly containing sodium selenite (1–5 years, 0.5 mg; 6–9 years, 1.0 mg); control children received a placebo.)

group	year	number of subjects	total cases	alive	turned latent	improved	turned chronic	died
control	1974	3985	54	27	16	9	2	27
	1975	5445	52	26	13	10	3	26
treated	1974	4510	10	10	9	0	1	0
	1975	6767	7	7	6	6	0	1
	1976	12579	4	2	2	0	0	2
	1977	12747	0	0	0	0	0	0

Table 2. Hair and staple food selenium levels (micrograms per gram): regional characteristics in areas of China affected (A) and unaffected (U) with Keshan disease

(Numbers in parentheses are numbers of samples analysed.)

area	hair	soybean	sweet potato	wheat	oats	rice
hill region (A)	0.097–0.123 (79)	0.020–0.034 (9)	0.0029–0.0096 (15)	—	—	—
coastal region (U)	0.234–0.331 (40)	0.042–0.074 (4)	0.075–0.089 (4)	—	—	—
mountainous region (A)	0.084 (20)	0.022 (3)	0.0027 (4)	—	—	—
plain (U)	0.204–0.228 (40)	0.041–0.081 (4)	—	—	—	—
non-salted region (A)	0.056 (20)	—	—	0.0053 (6)	0.0053 (5)	—
salted region (U)	0.170 (20)	—	—	0.0196 (6)	0.0395 (4)	—
surrounding area (A)	0.060 (18)	0.0057 (4)	—	—	—	0.0078 (5)
'safe island' (U)	0.114 (20)	0.025 (5)	—	—	—	0.0202 (6)

essential trace element for man. A study of blood selenium levels from many people in affected areas led the Chinese to conclude that the minimum adequate value lies in the range 0.03–0.04 μg/g. Further, calculations of the selenium requirement of human populations based on similar observations, led to the conclusion that 30 μg/day was the best estimate of the likely minimal adequate intake (Chen *et al.* 1980). This value is of the same order as the value of 20 μg/day obtained by Stewart *et al.* (1978) from human metabolic experiments with [^{75}Se]-methionine. The question arises, however, as to whether Keshan disease is caused only by a

dietary lack of selenium or whether other environmental factors such as heavy metals, an infective agent, or dietary lack of vitamin E also play a part. This question remains to be resolved because appropriate surveys of heavy metal contamination in areas in which the disease occurs have not been carried out, and the vitamin E status of populations in these areas has not been measured. It is, however, of interest to compare the selenium status of people with that of subjects in the Keshan disease areas, and values are available from at least three regions: New Zealand, the Federal Republic of Germany and the United States.

TABLE 3. HAIR SELENIUM LEVELS AND BLOOD GLUTATHIONE PEROXIDASE ACTIVITY IN POPULATIONS SUSCEPTIBLE TO KESHAN DISEASE

(Values given are mean values ± s.e.; numbers in parentheses are numbers of samples.)

population	hair selenium μg/g	glutathione peroxidase activity†
city children, Heilongjiang	0.390 ± 0.018 (20)	—
commune children, Heilongjiang	0.151 ± 0.011 (20)	—
city children, Sichuan	0.131 ± 0.014 (16)	—
commune children, Sichuan	0.069 ± 0.001 (10)	—
commune staff's children	0.238 ± 0.011 (20)	90.0 ± 1.2 (18)
commune peasant's children	0.128 ± 0.009 (20)	61.9 ± 2.8 (20)
mine staff's children	0.161 ± 0.007 (26)	80.2 ± 1.2 (23)
mine peasant's children	0.058 ± 0.003 (22)	60.5 ± 0.7 (63)

† Decrease in micromoles GSH per 5 min per 8 μl blood, corrected for non-enzymic oxidation of GSH.

The low dietary intake of selenium by many New Zealand residents has been investigated in Dunedin by Robinson and her colleagues. In a study of 264 New Zealanders, some of whom were resident in Auckland, where the soil selenium level is not considered to be low, and others from around Dunedin, which is a low selenium area, a close relationship was found to exist between blood glutathione peroxidase activity and blood selenium concentration (Thomson *et al.* 1977). Values ranged from 0.02 to 0.14 μg/ml whole blood, corresponding to glutathione peroxidase activities of 0.2–3.0 units/ml whole blood; above a blood selenium concentration of 0.10 μg/ml, the correlation with glutathione peroxidase activity was less good than when blood selenium was lower. The value of 0.1 μg Se/ml whole blood was 'a little above the mean' for New Zealand residents, but, despite the low level of blood selenium observed in many of the subjects tested, no pathological conditions were observed in the population under investigation, even in those subjects at the lowest end of the scale of values.

In Düsseldorf, Lombeck and her colleagues have investigated blood selenium and glutathione peroxidase levels in normal children, and compared these with children with maple-syrup urine disease or phenylketonuria and who were given diets containing mainly protein hydrolysates or amino acid mixtures as their amino acid source (Lombeck *et al.* 1978). Mean serum selenium concentrations in normal children were in the range 35–85 ng Se/ml, compared with those in the children with genetic disorders, which showed values ranging from 4–35 ng Se/ml. Erythrocyte glutathione peroxidase values for normal children were in the range 6.5–9.8 units/g haemoglobin, and for the dietetically treated children 3.1–5.9 units/g haemoglobin. The very low levels of serum selenium and of the selenium-dependent enzyme in the children with genetic disorders were not associated with any adverse signs apart from those associated with phenylketonuria and maple-syrup urine disease.

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Many values have been recorded for blood selenium levels of residents of the United States. A recent study by Levander *et al.* (1981) is of particular interest. A selenium depletion–repletion experiment was carried out in six healthy young male volunteers who were confined to a metabolic ward for 10 weeks and given a low-selenium liquid formula diet (19–24 μg Se/day), based on New Zealand casein, for 45 days, followed by the same diet supplemented to a level of 203–224 μg Se/day for a further 24 day period. Among the many measurements made were plasma selenium level and erythrocyte glutathione peroxidase. Mean plasma selenium concentrations decreased during the depletion phase from 0.136 ± 0.015 μg Se/ml to 0.097 ± 0.019 μg Se/ml and returned during the repletion phase to 0.142 ± 0.009 μg Se/ml.

TABLE 4. BLOOD (OR SERUM OR PLASMA) SELENIUM LEVELS (NANOGRAMS PER MILLILITRE) IN HUMAN SUBJECTS

Thomson *et al.* (1977) (whole blood)	Lombeck *et al.* (1978) (serum)		Levander *et al.* (1981) (plasma)	Chen *et al.* (1980) (whole blood)	
	dietetically treated children	normal children		Keshan disease area	'safe' area
20–140	4–35	35–85	97–142	5–10	20–50
mean 95	mean 28	mean 60	mean 136	—	—
calculated values for comparison (whole blood)					
20–140	6–52	53–130	145–210	5–10	20–50
mean 95	mean 42	mean 90	mean 200	—	—

Comparison of blood glutathione peroxidase levels in these three studies with those found in Keshan disease is not possible because different methods were used to measure enzyme activity. However, the methods used for selenium determination in the different laboratories were essentially similar, and it is reassuring to note that Mertz (this symposium) has reported remarkable agreement in selenium values obtained in different laboratories for the same test sample. Conversion of the serum selenium levels to whole blood selenium levels may be made by assuming that 60 % of the total selenium is present in the erythrocytes and 40 % in the serum or plasma (Thomson *et al.* 1977; Diplock, unpublished observations). Table 4 shows blood, serum or plasma levels of selenium in the above four studies, and these levels are given in the units used in the papers cited, and also calculated values for whole blood selenium level based on the literature values. It is evident that the blood selenium levels (5–10 ng/ml) in subjects from Keshan disease areas are much lower than any other recorded values, although it is noteworthy that some of those observed in the children with genetic disorders in Düsseldorf are in the same range. It can therefore be concluded that the major cause of Keshan disease is likely to be the exceptionally low selenium status of these subjects, although the possibility that low levels of vitamin E, high levels of heavy metals, or an infective agent may be involved, cannot be ruled out.

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Discussion

J. R. Todd (*Agricultural and Food Chemistry Department, The Queen's University of Belfast, U.K.*). Since very low selenium contents are recorded in foodstuffs of plant origin in the areas where Keshan disease occurs, it would be expected that corresponding myopathies would occur in domestic livestock. Have they been reported?

A. T. Diplock. Yes, conditions suggestive of mullberry heart disease in pigs and exudative diathesis in chickens have been observed in Keshan disease areas.

J. R. Todd. In muscular dystrophy in farm animals lesions may occur either in the heart muscle or skeletal muscle. In Keshan disease do lesions occur only in the heart muscle?

A. T. Diplock. No, there is also some evidence of muscular weakness in the legs of some of the patients.

I. Thornton (*Applied Geochemistry Research Group, Department of Geology, Imperial College, London, U.K.*). Professor Diplock has given an interesting account of the occurrence of Keshan disease found in rural communities in hilly areas along a belt through central China. He mentioned that this had been referred to as a geochemical disease. Does the known distribution relate to areas underlain by any particular geological material or geochemical facies and is there any information on the nature of the dominant soil types? Has the disease been clearly shown to be due to dietary deficiency of selenium, or are there possible contributory causes due to interactions with toxic heavy metals or metalloids such as zinc, cadmium and arsenic?

A. T. Diplock. The results of a geological study have been reported (Tang 1979) but a translation from Chinese has not yet been made. While all the evidence points to a clear dietary deficiency of selenium, as evidenced by the very low blood levels reported, the possibility of heavy metal or metalloid involvement cannot be excluded. The possibility of marginal deficiency of vitamin E also exists because of the widespread practice of salt-preservation of vegetables, which may destroy vitamin E.

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R. J. P. Williams, F.R.S. (*Wadham College, Oxford, U.K.*). I wish to give a possible explanation for the appearance of three different ways of eliminating peroxides in biology – namely through catalase, vitamin E and glutathione peroxidase.

Catalase eliminates H_2O_2 and *small primary* peroxides in water very rapidly but since it is an iron metalloenzyme it does so through one-electron reactions. The various oxidation states of FeO, must not be exposed to organic molecules for they could themselves initiate the very radical reactions that catalase is designed to prevent. In fact in this enzyme haem is buried deeply (M. Rossmann, personal communication). It is the steric hindrance to the active site that prevents catalase from clearing secondary, tertiary or long-chain primary peroxides.

Glutathione peroxidase is a selenoprotein. The Se atom is on the surface of the structure (Ladenstein *et al.* 1979). It is also a two-electron reaction centre. It can therefore clear peroxides without the danger of generating free radicals. It is a *slower* catalyst than catalase but a more general one.

Both catalase and glutathione peroxidase act in water. Vitamin E is in the membranes and works on membrane-soluble peroxides. Again it is really an exposed two-electron reactant, going through relatively inert radicals, and does not easily generate radical chains.

The efficient clearance of peroxides of different structures and solubilities requires a battery of catalysts of the kinds that have been found in biology.

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A. T. Diplock. I think that Professor Williams's explanation, in terms of accessibility of their active site, of the different substrate specificity of catalase and glutathione peroxidase is a very interesting one, which throws light on a difficult problem.

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