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Problems on Selenium in Animal Nutrition

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Problems on Selenium in Animal Nutrition

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Content

		Page
1.	Introduction **A. Frøslie**	5
2.	Relation of selenium to other antioxidants, with special reference to free radicals **B. Karlmark**	11
3.	Bioavailability of selenium **J. Hakkarainen**	21
4.	Acute and chronic selenium toxicity **M. Sandholm**	37
5.	Methods for the evaluation of selenium status **S. Sankari**	51
6.	Notes and comments on the determinarion of selenium in biological materials **V. Galgan & A. Frank**	57
7.	Analytical problems concerning glutathione peroxidase **S. Sankari**	75
8.	Diseases and diffuse disorders related to selenium deficiencies in ruminants **B. Pehrson**	79
9.	The pathology of diseases and diffuse disorders due to selenium deficiency in non-ruminants **L. Jönsson**	95
10.	Relations between selenium and immunity **H.J.S. Larsen**	105
11.	Optimum selenium levels in animal products for human consumption **J. Aaseth**	121
12.	Disorders related to selenium deficiency in man **A. Aro**	127
13.	General aspects of selenium fertilization **G. Gissel-Nielsen**	135

14. Selenium fertilization in Finland: selenium soil interactions
T. Yläranta 141

15. Selenium fertilization in Finland: selenium content in feed and foods
P. Varo 151

16. Selenium fertilization in Finland: effect on milk and beef production
L. Syrjälä-Qvist & P. Aspila 159

17. Effect of selenium on the health of the dairy cow, with special reference to udder health and reproduction
E. Jukola 169

18. Effects of selenium fertilization on the human selenium status and the environment
G. Alfthan 175

19. Optimization of the selenium flow to man
K. Edelmann 183

20. Selenium supplementation in pigs
H. D. Poulsen 189

21. Selenium supplementaton in ruminants
G. Øvernes 199

Posters

22. Selenium-dependent- and selenium-independent activity of glutathione peroxidase in the liver cytosolic fraction from three week-old chickens
C. Lauridsen & K. Jacobsen 205

23. Selenium content in milk during the selenium fertilization in Finland
P. Ekholm, M. Ylinen & P. Varo 211

24. Selenium of the selenium yeast enters the cow's milk
K. Suoranta, E. Sinda & R. Pihlak 215

1 Introduction

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Selenium is an essential trace element which can nevertheless be toxic. In the mid 1930s the element was identified as the toxic component of animal feed crops, which in certain parts of the USA caused chronic disease and death in grazing livestock (McDowell 1992). The disease has been known for centuries and it is claimed that Marco Polo was the first to describe the condition from his travels in China in 1295. However, the association between selenium and the diseases "blind staggers" and "alkali disease" was not discovered until fairly recent times. As far as we know, selenium poisoning as a result of high natural levels of the element in forage plants does not occur in the Nordic countries.

It was only the toxic effects of selenium which were known in the period from the mid-1930s up to the second half of the 1950s. It was then that Schwarz & Foltz (1957) first demonstrated the beneficial effects of selenium in preventing liver necrosis in rats. In other words, selenium was identified as a vitally necessary substance - an essential trace element.

Selenium has thus been known as an essential element for only 35 years - though in the short time up to the present day, it has undergone quite a metamorphosis. From being considered purely as a toxic element, it is today one of the most focused-upon essential trace elements in livestock husbandry and veterinary medicine. Selenium has also become the subject of considerable interest in human medicine.

The enzyme glutathione peroxidase was discovered the same year as the beneficial effects of selenium were demonstrated (Mills 1957), although the enzyme was not identified as a selenoprotein until 1973 (Rotruck et al. 1973; Flohé et al. 1973). It was only *then* that selenium's mode of action as an antioxidant was recognized, and its interplay with vitamin E, unsaturated fat, and sulphur-containing amino acids became apparent. Vitamin E had been known as the "antisterility vitamin" from the early 1920s (Evans & Bishop 1922), but it was not until the 1940s that its role as an antioxidant was revealed.

It did not take very long after 1957 before the significance of a lack of selenium for the now well-known and typical selenium deficiency diseases in farm animals was clarified, and selenium was taken into use in the prevention and treatment of these conditions (McDowell 1992). However, the authorities in many countries were hesitant to permit direct selenium supplementation of feed mixes, such as was the practice with other essential trace elements. This was no doubt due to selenium's past history as a toxic substance, and the fact that in the forties it had been considered to exert a carcinogenic effect in experimental animals. As late as 1967, the Norwegian Advisory Committee on Veterinary Drugs, Pesticides, and Feedstuffs stated that the element was too toxic to be added to feedstuffs. However, there is a sufficient margin between nutritional requirements for selenium and tolerance levels to allow the substance to be supplied to animals in the feed in a satisfactorily safe way. Feed manufacturers have developed methods to ensure an even

and correct mixture, and selenium addition to feed concentrates is now a fully accepted routine practice in countries where there is need for supplementation.

As was mentioned, diseases which have subsequently proven to be due to selenium deficiency have been known for many years. Fürstenberg (1864) described a disease in lambs similar to that now known to be associated with selenium deficiency. In Scandinavia, we have accounts of muscular degeneration in ruminants dating right back to the 1890s (Slagsvold & Lund-Larsen 1934). The disease was reported from Sweden by Hoflund in 1941 and in Finland by Broberg in 1949. Slagsvold & Lund-Larsen (1934) gave a detailed description of the disease in lambs and young cattle in Norway, and their paper from 1934 is still worth reading, even though the authors were of course unaware of the causal relationships. It is also interesting to note that Slagsvold (1925) described a disease which he called cod-liver oil poisoning, and which was undoubtedly due to selenium-vitamin E deficiency, and which was induced by intake of unsaturated fat. Not least is this interesting in the light of the fact that diets high in unsaturated fat are now fed to selenium-vitamin E deficient livestock in order to obtain animal models for the study of selenium-vitamin E deficiency conditions.

Following developments in Scandinavia a little further, there is every reason to mention Anna-Lisa Obel's work from 1953 on so-called liver dystrophy in pigs. She discovered that the disease could be prevented by giving sulphur-containing amino acids. It was a breakthrough when the condition could be termed "hepatosis dietetica" - nutritional hepatosis - even though Obel did not completely fathom out the cause.

After 1957, there was a considerable upswing in research into selenium-vitamin E deficiency diseases in farm animals, not least in the Nordic countries. This was especially the case in Sweden and Finland, where, from the 1950s onwards, a great deal of research of international interest was carried out. As early as 1962, Aas Hansen published a review article in the Journal of the Norwegian Veterinary Association on the prophylactic and therapeutic use of selenium in muscular dystrophy. This article cites several of the Nordic pioneer studies on selenium deficiency. Time does not permit me to go into further detail now, and I must therefore refer you to textbooks and review papers on the subject. I will, however, mention the study by Andersson (1960), in which he demonstrated hypofunction of the thyroid gland by a histometric method in young cattle suffering from nutritional muscular dystrophy. These findings are interesting in connection with the recent discovery by Berry et al. (1991) that selenium is a part of the enzyme that catalyses the thyroid hormone into its active form.

The increasing availability of analytical methods has also gradually made it possible to map the selenium status of livestock and feed plants in the Nordic countries. Extreme deficiencies of selenium in pasture plants and feed cereals were found in large parts of Scandinavia, including Finland (Mattsson 1982). To alleviate this situation, Finland, as the first country in Scandinavia to do so, began to add selenium to feed concentrates in 1969. Denmark followed suit in 1975, Norway in 1979, and Sweden in 1980. Vitamin E supplementation of feed mixes for pigs and poultry, and to some extent also of feed for ruminants, is now standard practice. Moreover, selenium-enriched artificial fertilizers were introduced in Finland in 1984 to increase selenium levels in cereals, vegetables, and forage crops.

One might presume that all our problems concerning selenium intake in livestock have

now been solved, and that today's symposium is perhaps superfluous. Not at all. There are still a number of questions remaining to be answered as regards selenium and vitamin E in animal nutrition, especially problems connected with the more diffuse disease conditions attributed to deficient or marginal intake of selenium. We also lack knowledge of the practical significance of selenium's effect on the immunological defence systems, prostaglandin synthesis, the synthesis of thyroid hormone, and detoxification of heavy metals such as mercury and cadmium. Nor do we know enough about the metabolism, bio-availability, and biopotency of the various organic and inorganic forms of selenium used as supplements. Requirements under various conditions, and the risk of overdosage represented by the different routes of administration are also aspects which have not yet been satisfactorily clarified. Finally, we have the environmental and human nutritional issues associated with the incorporation of selenium in artificial fertilizers. These have also not yet been fully elucidated, and are moreover very controversial.

There are thus many questions arising in connection with practical livestock nutrition, and I shall mention a few of the major ones.

- How great is the practical requirement of livestock for selenium in the feed under different husbandry conditions? What are the tolerance levels? Is the difference between organic and inorganic selenium so great that we should define requirements and tolerance levels according to the selenium source?

- What are the deficiency and tolerance limits for selenium in samples from livestock, what organs should we analyse, what should we determine and by which methods?

- Should we supply selenium in the feed where needed, and if so, in what amounts and in what form? Should we continue to use "non-physiological" inorganic selenium as feed supplement, or should we give organic selenium compounds? If so, what doses should be employed?

- Should we add selenium to the soil in areas where it is deficient, so that selenium is taken up and incorporated as organic selenium in forage plants? If so, how much should we spread on the soil to cover livestock and human requirements without adversely affecting the environment? Is there a danger of overdosing livestock?

- Should measures be based on giving supplements to individual animals or individual herds when insufficient selenium is otherwise supplied in the feed? If so, how can this best be achieved? Should we use mineral mixes or other feed supplements, injectable depot preparations, or rumen pellets?

We will be discussing these questions and many others during this symposium, as well as considering what basic knowledge we possess, and what we lack and need to obtain, in order to be able to answer them.

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2 Relation of selenium to other antioxidants, with special reference to free radicals

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Research activities on free radicals in biology have created an abundance of possibilities for defeating their attacks on vulnerable living tissue. Besides the natural antioxidant mechanisms, there are also supplementary possibilities such as vitamin A, vitamin C, vitamin E, selenium, carotenes, retinols and coenzyme Q10, which are already proving to be promising as prophylactic as well as therapeutic agents. It is not yet possible, however, to grade their respective importance under different circumstances. A large number of pharmacological approaches are also under development for defeating or, in some cases, creating free radicals.

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BACKGROUND

Four billion years ago the photosynthesis process started to produce oxygen on earth. A global "catastrophy" had been initiated, somewhat comparable to a hypothetical situation where steadily increasing chlorine gas production might be started today (Gilbert 1981). This oxygen threat forced the microorganisms to escape along two alternative pathways. One route was a move to environments without oxygen and those victims became the ancestors of our anaerobic microorganisms. The others adapted to the new oxygen-rich environmental conditions and learned how to use oxygen in a positive way. Our ancestors used this second alternative.

We have learned, however, to breathe a gas containing around 20% oxygen. Higher concentrations are toxic, which was already known some 200 years ago. The discovery of the enzyme SOD (superoxide dismutase) some 20 years ago led to the conclusion that the oxygen toxicity was due at least partly to formation of oxygen radicals.

More than four scientific works are published per day, per annum, on free radicals and antioxidants and the purpose of this overview is not to present original data but to give a sampling of the vast scientific work which is presently devoted to these issues.

FREE RADICALS

Free radicals are characterized by having a single unpaired electron in the outer orbital

instead of paired electrons; the latter is the natural and chemically stable condition. The free radicals (R1 and R2) easily take an electron from (or give to) another molecule (M), which continues to take or give its unpaired electron further.

$$R1{:}R2 \rightarrow R1\cdot + R2\cdot$$
$$R1\cdot + M \rightarrow R^{+} + M\cdot$$

This chain reaction continues until two free radicals meet and neutralize their respective reactivities.

There are different kinds of free radicals. One example is nitrous oxide (NO), which has recently been described as an endogenous free radical with physiological vasodilator properties (Palmer et al. 1987). Other most frequently studied free radicals are the oxygen radicals. While NO is regarded more as the oil in a biological machinery, the oxygen radicals can be regarded as endogenous coarse sand in the metabolic machinery formed under "oxidative stress".

The oxygen radicals are formed in the following reactions:

$$O_2 + e^{-} \rightarrow O_2^{-}\cdot$$
$$2O_2^{-}\cdot + 2H^{+} \rightarrow H_2O_2 \text{ (catalysed by SOD)}$$

The continuous decomposition of H_2O_2 could take two main routes:

(a) $H_2O_2 + 2e^{-} + 2H^{+} \rightarrow 2H_2O$ (catalysed by peroxidases and catalase)
This is the preferable and "harmless" reaction.

(b) Hydrogen peroxide receives an electron from a metal complex (like Fe^{2+}), and the following reaction takes place:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + OH\cdot$$

The hydroxyl radical (OH·), which is the end product in the chain described, is the main reactive destructor in biology. It destroys hyaluronic acid, disintegrates collagen and attacks cells.

PHYSIOCHEMICAL MECHANISMS FOR PRODUCTION OF FREE RADICALS

Homolysis

A covalent bond is broken to give one electron each to the two moieties (see above).

Radiolysis
Radioactive irradiation as well as X-rays are known to split water under the formation of free radicals. The cell damage which follows might, however, in selected cases, also be of value in the treatment of, e.g., malignancy.

Photolysis
Photosensitizers, like riboflavin, bilirubin, porphyrines and pharmaceuticals like phenothiazines, sulphonamides and diuretics absorb energy which excites oxygen to a highly reactive radical. UV- as well as infra-red light have also been shown to induce free radicals. Cataract of the lens in the eye is a well-known negative effect with this background.

Redox processes
These processes include electron transfers which can create as well as neutralize free radicals. Adriamycin is an example of an exogenous compound which creates free radicals and which is also therapeutical for a large variety of malignancies.

PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL PRODUCTION OF FREE RADICALS

Free radicals are involved in a great many different mechanisms which lead to pathological changes in tissues and organs. Since the hydroxyl radical is so highly reactive, there is reason to believe that the injury it causes is located in the near vicinity of its formation. Since divalent iron (Fe^{2+}) is one major component in the formation of free radicals, the destruction is often limited to molecules containing iron. Most of them are easily replaced in the cell, however. The fate is worse for metal-sensitive parts of the DNA, which might be irreversibly damaged with deleterious effects on the genetic code.

Cell metabolism
The respiratory chain in the mitochondria normally produces oxygen radicals in limited amounts. If this chain is weakened by external or internal influences, however, the antioxidant enzymes can no longer fulfil their tasks and an unhealthily large amount of free electrons will attack cell metabolism.

Lipid peroxidation
The most common injury caused by free radicals, however, is lipid peroxidation. Polyunsaturated fatty acids are attacked by free radicals causing a subtraction of hydrogen leading to formation of a lipid radical. This easily reacts with oxygen to form a peroxy-radical, lipid-$O_2\cdot$. This, in turn, binds a new hydrogen from another polyunsaturated lipid and the chain of reactions continues. In this way lipids are continuously destroyed, but the sequence of reactions can be broken, provided the availability of the selenium-containing enzyme glutathione-peroxidase (GSH-Px) is sufficient.

The normal ageing process probably includes lipid peroxidation. This theory is supported by findings that animals with a high relative oxygen consumption seem to have a shorter life. Additionally, their life span seems to increase with the addition of anti-

oxidants. Moreover, the occurrence of SOD in these animals seems to correlate positively with the life span of these animals as well (Rowe 1992). The more SOD, the longer the life.

The development of atherosclerosis consists of mechanical (hypertension!) as well as chemical factors. The muscular cells in the vessels are also continuously attacked by free radicals, with oxidation of LDL (low density lipoproteins) as a result. This LDL is deposited in the intima layer of the vessels. A centripetal thickening and sclerosation of the walls of the vessels prevents, furthermore, the nutrition as well as the beneficial action of SOD (Nilsson 1993). There is today substantial evidence from animal studies, epidemiological studies as well as from double-blind clinical studies that the addition of antioxidants are of benficial value in the inhibition of the atherosclerotic process.

Mutation injuries

DNA/RNA are also damaged by free radicals. The subsequent change in proteins, enzymes and hormones might be deleterious for the whole organism. Development of some malignancies through this route is frequently discussed. In the 1960s WHO (Environmental Health Criteria) proposed that 75% of all cancers in man were caused by environmental factors. To day this figure is thought to be closer to 90% and the causes are to be found in exogenous as well as endogenous factors. Free radicals of different kinds probably play a major role in the induction of cancer and there are good reasons to believe that an intake of antioxidants (see below) is of prophylactic as well as therapeutic value.

Auto-oxidation

A direct formation of superoxide radicals can occur in reactions between oxygen and catecholamines, reduced iron and others. One example is also the haemoglobin molecule which normally binds oxygen to its divalent iron (Fe^{2+}) in the protein. If, however, the protein stucture is changed, as in a reduction of pH, the oxygen will oxidize the iron to its trivalent state, which leads to the formation of hydroxyl radicals as described.

Neutrophils

During phagocytosis of microorganisms by neutrophil granulocytes, oxidative enzymes are released which produce large amounts of superoxide radicals which, in turn, are transformed into oxygen, hydrogen peroxide and hydroxyl radicals (see reactions above). The granulocytes then, like a machine gun, attack with electron bullets the encapsulated microorganism until total destruction. It is also possible that superoxide leaks from the neutrophils and acts chemotactically for other defensive neutrophils and macrophages.

Ischaemia - reperfusion

An impairment of oxygen flow to an organ creates a cellular inflow of Ca^{2+} which, in turn, activates xanthine-oxidase, giving rise to an increased production of hypoxanthine (Fig. 1) from ATP, ADP and AMP, but the lack of oxygen (ischaemia!) prevents the hypoxanthine from being further metabolized to xanthine.

When the reperfusion starts with an instantaneous supply of oxygen, a rapid metabolism of hypoxanthine takes place with formation of a torrent of xanthine and oxygen radicals.

Muscular tissue does not contain xanthineoxidase, which renders it a lower risk of developing free radicals. This fact has clinical correlates. First, the muscular ischaemia during prolonged anaerobic metabolism is hardly vulnerable for the athlete and, secondly, a torniquet on a limb during surgery can be used for a long time to facilitate the surgeon, but ischaemic injuries are seldom seen at reperfusion.

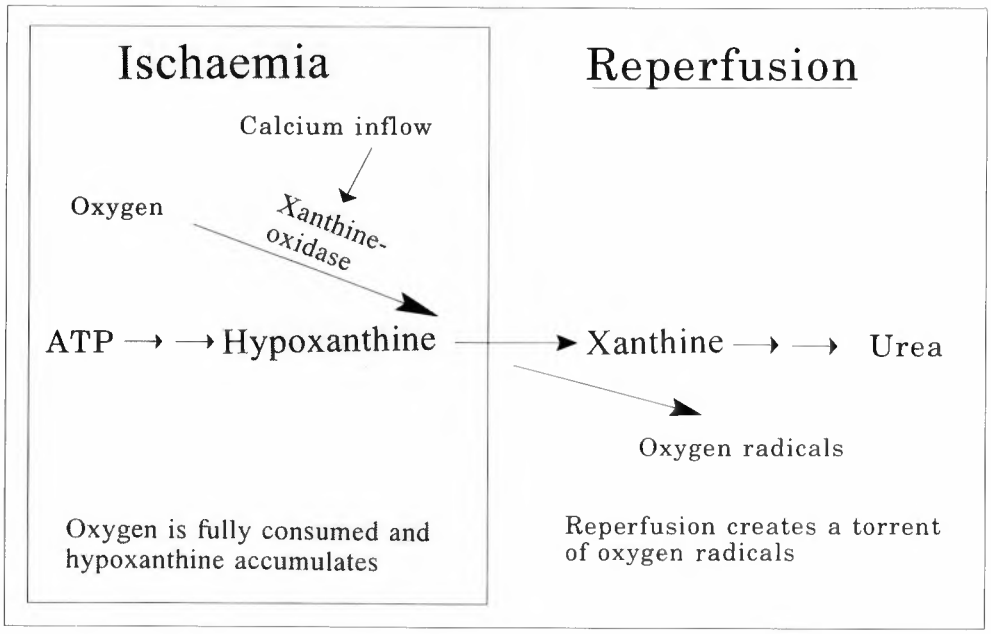

Fig. 1. Metabolic pathways which create a momentary excess of oxygen radicals at reperfusion after ischaemia

DISEASES AND DISORDERS FROM FREE RADICALS

Free radicals are considered to be pathophysiologically involved in more than 200 diseases and disorders which are a part of civilized life. Among them are the following (Mögelvang 1992):

Arteriosclerosis
Myocardial infarction
Cancer
Parkinson's disease
Emphysema
Alcohol-induced cardiomyopathy
Ozone poisoning
Alcohol overconsumption
Stroke
Ischaemia-reperfusion syndrome
Senile dementia
Rheumatoid arhtritis
Some autoimmune diseases
Heavy metal poisoning
Tobacco overconsumption

There is a long way to go before the exact role of free radicals can be established as components in the genesis of the diseases mentioned and a successful pharmacological programme in the treatment of these diseases will take even longer.

DEFENCE AGAINST FREE RADICALS

There are a large number of defence mechanisms against free radicals, and these can be classified as natural defences and scavengers. Some of the most frequently investigated ones will be mentioned, but the list is steadily growing.

Natural defences

Superoxide dismutase (SOD)

This enzyme catalyses the conversion of the superoxide radical, $O_2^{-}\cdot$ to H_2O_2, thereby inhibiting the former from reducing the naturally occurring Fe^{3+} to the more "toxic" Fe^{2+}. It must be borne in mind, however, that the action of SOD leads to an increased production rate of H_2O_2. This compound is, in turn, eliminated by a powerful action of the selenium-containing enzyme GSH-Px.

Selenium-dependent GSH-Px

At least five different kinds of selenoproteins are identified. Three of them are peroxidases, one is selenoprotein P and one is a deiodinase which catalyses the conversion of thyroxine to triiodothyronine in several organs (Levander 1992).

Only a minor part of selenium in the human body is fully identified and it is quite understandable that our knowledge about relations between the selenium intake, plasma concentrations of selenium and antioxidant status is limited. Hopefully, the present symposium will cast further light on this medical conundrum.

The GSH-Px catalyses the conversion of peroxides to the corresponding hydroxy compounds. Not only the H_2O_2 is catalysed to the hydroxyl radical but also steps in the lipoxygenase phase of the metabolism of arachidonic acid. It catalyses the conversion of 5-HPETE (precursor to the leucotrienes) to HETE.

High oxidative stress through addition of polyunsaturated lipids is shown to be counteracted to a certain extent by the addition of selenium to the food (Committee on Diet and Health 1989). Additionally, supplementation with high doses of selenium to animals has indicated a reduced risk for development of malignancies.

Selenium also works as an alternative antioxidant when bound as selenomethionine and selenocysteine. By its dual action, then, selenium seems to be an important compound in the defence against oxidative stress (Mögelvang 1992).

Selenium-independent GSH-Px

To make the role of GSH-Px even more complicated, it was found that there is a selenium-independent GSH-Px, which was identified as glutathione S-transferase B (Lawrence et al. 1978). The activity of this enzyme increases during selenium deficiency and may play a partial compensatory role for the loss of selenium-dependent GSH-Px, which occurs during low selenium conditions. Despite this, there seems to be a linear relationship between

concentration of selenium in plasma and the GSH-Px activity, which should be interpreted as a minor role for the selenium-independent GSH-Px, at least in the body as a whole.

Transferrin
The cell needs ionized-free iron for different synthesis processes in its inner milieu and cells are, accordingly, vulnerable to formation of free radicals. The intracellular enzymes SOD, GSH-Px and catalase offer good protection, however. Since these enzymes are lacking in the extracellular space, other protective mechanisms exist, one of which is transferrin which very efficiently binds all free iron ions, thus preventing them from participating in the formation of free radicals.

Xanthine oxidase antibodies
Xanthine oxidase might leak to the extracellular space and activate formation of oxygen radicals, but blood plasma contains antibodies specifically directed towards xanthine oxidase, thereby minimizing the formation of hydroxyl radicals by this route.

Antioxidants as scavengers
Vitamin E
The most obvious action of the vitamin is as a scavenger of free oxygen radicals, although a large number of other important physiological actions are also described. Vitamin E is one of the most important lipid-soluble factors in preventing the peroxidation of low-density lipoproteins (LDL). This prevention is also shown to counteract the development of atherosclerosis (Holm 1992). Vitamin E is, thereafter, rapidly regenerated with the help of other antioxidants, such as vitamin C.

Pre-treatment with vitamin E before heart by-pass operations has been shown to reduce the concentration of hydrogen peroxide which occurred in control patients after start of the reperfusion (Holm 1992).

Vitamin C
Vitamin C is probably the most important water-soluble natural antioxidant. It reacts directly with the hydroxyl radical, but is also shown to be able to reduce oxidized vitamin E.

Coenzyme Q_{10} (Ubiquinon)
Coenzyme Q_{10} is less well studied as an antioxidant, although it shows antioxidative properties *in vitro* as well as in intact animals (Beyer 1989).

It is a coenzyme in the electron transport chain of the mitochondria and participates in the formation of ATP. The coenzyme is produced in parallel with cholesterol from acetyl-CoA and any increase in production of one of them automatically increases the production of the other. There is therapeutic trend in human medicine to use cholesterol-reducing drugs, but such an approach must also be regarded as a threat to the normal antioxidative role of coenzyme Q_{10}. Such treatment will probably also reduce the synthesis of the coenzyme.

Carotene and retinol

There are indications that retinol (vitamin A), carotenes (provitamin A) and different synthetic carotenoides (more than 500 are known) might have a more specific protective effect against cancer, which at present cannot be distinguished from their antioxidant effect. Early studies did not, however, differentiate between these different compounds and conflicting results have therefore been obtained. The overall interpretation of the results indicates that a *deficiency* of vitamin A enforces a coexisting cancerogenic potential and that retinoids might have a preventive effect.

Earlier studies were focused on the role of different carotenes as precursors to retinol, but lately their own antioxidant effect is studied. Paradoxically they seem to be more potent as antioxidants at low oxygen pressures compared to most other antioxidants which mainly have their benefits during high oxygen pressures (Bruce 1990). In general, vitamin A is not thought to counteract the formation of lipid peroxidation.

Comparisons between antioxidants

A comparison was made in the rat between dietary β-carotene, coenzyme Q_{10}, vitamin E and selenium in their respective antioxidant power (Zamora et al. 1991). Erythrocytes as well as plasma were used as a test medium and a battery of tests were carried out. The conclusion was that the antioxidative properties in eryhtrocytes by β-carotene and coenzyme Q_{10} were quite comparable with those of selenium and vitamine E.

There is a discrepancy, however, between the recommended doses of antioxidants and the doses usually used in scientific work (Mögelvang 1992). In table 1, the different doses are described.

Table 1. Comparison between protective dose against free radicals, recommended dose and maximally non-poisonous dose for the most commonly used exogenously administered antioxidants

Antioxidant	Protective dose	Recommended dose	Maximal non-poisonous dose
Vitamin E (mg)	200- 1 000	10	3 200
Vitamin C (mg)	200 - 1 000	60	5 000
β-Carotene (mg)	20 - 40	6	180
Selenium (μg)	125-200	125	1 000

Table 1 indicates a discrepancy between the need for antioxidative intake and the recommended intakes of today. There is reason to believe that the need for antioxodants will increase in the future, since the oxidative stress, including the burden from the environment, seems to be on the increase. At the same time our intake of free radical scavengers seems to be decreasing in parallel with the development of civilization.

Other pharmacological approaches

A great deal of pharmacological work is being carried out to combat free radicals in medicine. Several models are used, but one of the most obvious conditions which lead to

formation of free radicals is shown in Fig. 1. Ischaemia and reperfusion occur daily in the clinics, for example in cases of myocardial infarction with acute thrombolytic treatment, during surgical treatment with angioplasty and in the course of the increasing numbers of organ transplantations. A large number of pharmacological studies are presently taking place to find remedies or prophylactic agents against free radical formation. Pharmaceutical companies are involved in a gigantic race.

SOD has hitherto been most commonly used. The human enzyme has now been cloned and can be extensively produced. Results are obtained from studies where SOD is added to the reperfusion fluid after ischaemia. The organ damage seems to decrease in some studies (Kloner 1989), but convincing and reproduceable clinical data are not yet available.

Desferrioxamine is another approach. This chelating drug binds iron and forms ferrioxamine which is rapidly eliminated through the kidneys. The iron molecule in haemoglobin, myoglobin, catalase, peroxidase and cytochrome is not touched by desferrioxamine, however, which increases the attractiveness of this remedy. EDTA (ethylene-diamine-tetra-acetic acid) is another metal-chelating compound which is being tried as a scavenger of free radicals in clinics.

Antiinflammatory drugs like NSAID have also been tried in the prevention of free radical formation in experimental ischaemia/reperfusion, but only equivocal results have been achieved.

The anti-gout drug allopurinol inhibits the formation of uric acid by inhibiting the enzyme xanthine oxidase (Fig. 1). This approach has been used several times and the results indicate a promising remedy.

Ebselen (PZ-51) is a new class of selenium which contains antiinflammatory agents that exhibit GSH-Px-like activity (Sies 1989). Ebselen is designed to mimic the active site of GSH-Px and the compound has been shown to possess antioxidative properties against lipid peroxidation in laboratory animals.

Quingaosu is an old Chinese antimalaria remedy which acts by creating free radicals. Its efficacy is enhanced by vitamin E deficiency (as expected) but not by a deficiency in dietary selenium. Supplementation of vitamin E to mice treated with quingaosu abolished its antimalarial action (Levander 1992).

A great many therapeutic efforts is being made to beat (or create) free radicals and we can expect a flood of increased knowledge within the next couple of years. Selenium is only one component in this gigantic puzzle.

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3 Bioavailability of selenium

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In order to guarantee the optimal health and production of domestic animals, it is of vital importance to have reliable information about the bioavailability of selenium. Use of the selenium function and the prevention of nutrient-specific disease assays provide a good estimation of the biological activity of selenium, whereas using the tissue level approach rather illustrates the passive biostorage of selenium. However, the latter test provides complementary information, especially with respect to optimal human nutrition. The evaluation of the biopotency of selenium using all these methods always implies the provision of average figures which are more or less bound to the experimental prerequisites. Unfortunately, the difference between the optimal and toxic dietary level of selenium is alarmingly small. There is an acute need for more research about the bioavailability of organic in comparison to inorganic selenium, with special reference to immunological and toxicological aspects.

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It is wellknown that selenium and vitamin E deficiency can have very serious consequences in the form of disease among domestic and experimental animals. Nutritional muscle degeneration, liver dystrophy, damaged vascular endothelium leading to capillary bleeding and oedema, microangiopathy (MAP) and exudative diathesis are among the most known pathological symptoms of these diseases. Studies of and research about the prevention of these deficiency states, have shown, among other things, that the biological activity of the different selenium compounds can vary between very wide limits. In fact, studies of the bioavailability of selenium started as early as 1958, shortly after the recognition of the nutritional essentiality of this trace element to animals (Schwarz & Foltz 1957; Patterson et al. 1957; Schwarz et al. 1957), when the pioneers Schwarz and Foltz evaluated the protective effects of various selenocompounds against liver necrosis in rats.

The basic discovery 15 years ago of the importance of free radicals in the aetiology of pathological disease states was the start of a new era, arousing the curiosity of medical scientists about the effects of selenium and vitamin E (Halliwell & Gutteridge 1989). As a result, during the past decade there has been a renewed interest in the bioavailability of dietary selenium, with special reference to the significance of animal assay data for human nutritional requirements. Several excellent reviews on this topic have been published (Combs & Combs 1986a; Mutanen 1986, 1987; Hassan 1987a).

In general, the information in the literature concerning the bioavailability of selenium can easily be characterized as being equivocal and even contradictory. In any case, the amount of data at first sight is so rich and diffuse, that an extemporized interpretation of the results is still difficult.

METABOLISM OF SELENIUM

Selenium is a paradoxical element. It may be defined as either a metal or a non-metal, a conductor or a non-conductor, amorphous or crystalline, colorant or decolorant, and a hydrogenator or a dehydrogenator. In nature, it may be found as metallic selenium and as inorganic or organic compounds.

Metallic selenium is non-water soluble and therefore of little use for plants and animals. The inorganic compounds of the element, sodium selenite and sodium selenate are widely used in animal selenium bioavailability tests. Sodium selenite is conventionally used as a reference substance in the studies of bioavailability.

The selenium present in cereal grains is mainly in the form of selenomethionine (Olson et al. 1970), whereas other forage plants also contain selenate and hitherto unidentified selenium compounds. Feeds of animal origin, such as fish- and meatmeal as well as milk powder, contain organic selenium since selenium is bound to proteins via amino acids. The composition of these selenocompounds are not known in detail for all feedstuffs. The selenium-rich fishmeal often contains, besides the organic compounds, selenium bound to heavy metals. Selenocysteine has been detected in mammalian tissues, in the enzyme glutathione peroxidase and in other selenoproteins.

The absorption of selenium from the gastrointestinal tract depends on its chemical form. Selenium absorption takes place via a normal concentration gradient, without any specific regulation mechanism. However, the l-form of selenomethionine is also absorbed against the concentration gradient, probably similar to that of l-methionine, that is, active transport is involved (Combs & Combs 1986b). Among ruminants, the absorption of inorganic selenium is significantly poorer than among single-stomached animals. This is probably due to the microorganisms in the rumen, which reduce selenium to an insoluble form. The rumen microorganisms can also form organic selenocompounds and incorporate selenium into bacterial proteins.

The inorganic compounds in the body are reduced to hydrogen selenide (H_2Se) and excreted as methylselenides or metabolized to selenocysteine and possibly to other amino acids. Hydrogen selenide can also bind with other metals and form metal selenides or protein compounds of these (Ganther 1984).

The metabolism of selenomethionine and selenocysteine is the best known among the organic selenium compounds. Selenomethionine is metabolized like methionine and here selenium functions analogously to sulphur. The selenomethionine present in nutrients and feedstuffs can be directly used for polypeptide and protein synthesis, or may be catabolized to selenocysteine and further to selenide. Selenomethionine may also be metabolized via transamination and decarboxylation to selenide.

Glutathione peroxidase (GSH-Px) is hitherto the best documented and well-known biologically active selenocompound found in animals. The role of selenium in the enzyme

GSH-Px was first demonstrated by Rotruck et al. (1973) and Flohé et al. (1973), who demonstrated that the biochemically active centre of this enzyme contains selenocysteine. It may be that selenocysteine is directly incorporated in the enzyme via translation; however, not all of the details about the binding of selenium to this enzyme are known.

At present, several isoenzymes with GSH-Px activity are known. Among the intracellularly active isoenzymes are the classical selenium dependent GSH-Px and a non-selenium dependent GSH-Px (glutathione transferase; GSH-Tr), as well as the selenium containing phospholipidhydroperoxide GSH-Px, which, differing from other enzymes, directly affects phospholipidhydroperoxides (Halliwell & Gutteridge 1989). A special selenium containing secretory glucosylerated GSH-Px is found in the extracellular space.

Recently, the presence of another selenium-dependent enzyme in mammals has been detected, namely the Type I iodothyronine deiodinase (Arthur 1991). This enzyme converts the inactive T_4-hormone to active T_3-thyroid hormone and it is also a selenocysteine-containing enzyme (Berry et al. 1991).

In addition, over the past several years a number of other selenoproteins have been isolated and even identified among animals, plants and bacteria, but definite knowledge about their function is still missing. As Sunde (1984) states "there are selenoproteins looking for a function and functions looking for a selenoprotein".

It is tempting to conclude that of all the above-mentioned more or less known selenocompounds, glutathione peroxidase constantly appears to be the only physiologically critical type known to date. However, this does not mean to say that there is no more research to be done. Indeed, there is much to do before all the biological functions of selenium in mammalian tissue are ultimately clarified. Whether or not the presently unknown selenocompounds are as critical for life as glutathione peroxidase, is entirely another matter; surprisingly, many may prove to be physiologically inert, serving only as selenium reserves for metabolic purposes.

We may also say with respect to the overall utilization of dietary selenium, that it is obviously a net result of several physiological and metabolic processes which convert a portion of ingested selenium to certain metabolically critical forms necessary for normal physiological function (Combs & Combs 1986a). The quantitative description of this biological utilization has come to be called the bioavailability (biological activity) of selenium.

METHODS OF ESTIMATING THE BIOAVAILABILITY OF SELENIUM AND SOURCES OF ERROR

One or more of the following biological methods are used to determine the bioavailability or biopotency of selenium.

Studies of how different forms of selenium prevent the occurrence or cure of specific deficiency diseases due to selenium deficiency: the criteria for physiological levels in research animals are in this case freedom from previously known pathological manifestations. Diseases which have been used in these types of studies are: the prevention of exudative diathesis in the vitamin E and selenium-deficient chick; the prevention of hepatic necrosis in the vitamin E- and selenium-deficient rat; the prevention of pancreatic atrophy

in the severely selenium-deficient and vitamin E-fed chick; and, finally, the more seldomly used prevention of dystrophies or myopathies in vitamin E- and selenium-deficient pigs, lambs, calves, or turkey poults. This type of assay could be called a preventive or remedial approach.

Measurement of glutathione peroxidase activity: For this variant, it is often a question of restoring normal GSH-Px activity in different tissues in animals having a depletion period-evoked selenium deficiency. This method can also be called a functional assay approach, and may even be carried out with human subjects using plasma or platelets as a source of GSH-Px activity.

Measurement of the uptake of selenium in different tissues, that is, selenium retention after the ingestion of selenocompounds or selenium containing feed/food: This assay involves the evaluation of the relative efficacy of known amounts of selenium in supporting the concentrations of that trace element in various tissues. The method may be used with any species, including humans. Of course, it may also be made on individuals who do not demonstrate any pathological signs of deficiency.

As mentioned previously, sodium selenite is the most frequently used standard in bioavailability studies. The calculation of the relative bioavailability may be made with one-point, point-slope or slope-ratio methods with each method having its disadvantages and advantages (Mutanen 1986).

It is known, that besides the deficiency of selenium and vitamin E, the feed content of sulphuric amino acids, polyunsaturated fatty acids, antioxidants, oxidative stressors and antimetabolites contribute in no small way and quite often to a determining extent to the occurrence of selenium deficiency diseases. It is clear that these factors will also interfere with selenium bioavailability studies, especially those based on the prevention or cure of specific diseases. The existing vitamin E and selenium status of the experimental animals and the dietary levels of vitamin E, methionine, β-carotene, vitamin A, ascorbic acid, ethoxyquin, protein, fat - especially with its content of polyunsaturated fatty acids (PUFA) - are good examples of possibly interfering factors (Hakkarainen 1984; Mutanen 1986, 1987; Hassan 1987a).

Where methods other than the prevention of disease are used to determine bioavailability, there are even more factors which may interfere with the metabolism of selenium in the body and thus either increase or decrease its bioavailability. The well-known interaction of selenium with heavy metals may decrease selenium utilization (Whanger 1981).

There are species differences in the results of the various assays; even age, sex and pregnancy may have an influence. Whether the experimental animals grow or do not, as well as the growth rate, can have a totally decisive influence on the results.

One of the most significant factors affecting the results and conclusions of a selenium bioavailability assay is the method used for the assay. This includes the animal species, the criterion of bioavailability, the method of measurement and the calculation methods.

In general, it may be concluded that a long series of experimental conditions can affect the determination of selenium bioavailability. Thus particular care must be taken with respect to this problem, at both the planning stages and the interpretation of achieved results (Hakkarainen 1984).

BIOAVAILABILITY OF SELENIUM

As mentioned in the introduction, the literature with respect to the bioavailability of selenium is very extensive; even this factor alone makes it very difficult to refer to the original reports in the present brief overall view of the subject. In addition, the literature is richly supplied with contradictory information. However, the picture of selenium's bioavailability is becoming clearer and more precise in the work published over the past two decades.

The relatively extensive information on hand about the bioavailability of discrete selenocompounds and the factors affecting the utilization of selenium from those compounds appears to be quite useful, especially since these selenocompounds are often used for selenium supplementation. On the other hand, this information is not as useful and as clear for the practical use of natural food- and feedstuffs as selenium sources.

It can be determined in practical animal experiments that sodium selenite both increases the activity of GSH-Px in the blood and prevents and cures deficiency diseases much more efficiently than the selenium found in selenomethionine, grains, and meat- and fishmeals. Just as convincing is the total evidence that the organic selenium sources are considerably more effective than the inorganic, if selenium bioavailability is used as a tool to increase the levels in animal tissue and, most importantly, meat.

As an example of the above-mentioned conclusions may be noted the results from investigations carried out at our Department of Clinical Nutrition, Faculty of Veterinary Medicine, Uppsala, in collaboration with the Department of Biochemistry, College of Veterinary Medicine, Helsinki, where, over the past several years, we have devoted a great deal of effort to determining the bioavailability of selenium in standard feed, wheat, barley, oats, fishmeal, meatmeal and selenomethionine (Hassan 1986, 1987b; Hassan et al. 1987a, 1987b, 1990, 1993). The studies have been carried out on chicks, and the following criteria for the effect of selenium were used: the activity of the selenium-dependent enzyme glutathione peroxidase in blood plasma and in whole blood; the ability to prevent the selenium deficiency disease exudative diathesis; and the concentration of selenium in various tissues.

The results shown in Table 1 demonstrate that the biological value of selenium varies significantly in the investigated selenium sources. A clear difference between the results obtained using GSH-Px measurement and the prevention of exudative diathesis on the one hand and the tissue concentration on the other hand may also be observed. Using GSH-Px measurement and the prevention of exudative diathesis with the inorganic salt selenite as a reference substance (100%), gave the following mean values for selenium bioavailability for the different products: wheat, about 85% (83-100%); barley, about 80% (78-85%); oats, about 45% (45-41%); fishmeal, about 65% (64-80%), meatmeal, about 25% (22-30%); and selenomethionine, about 75% (77%). For example, these values mean that the chick body will be able to utilize about three times as much of the selenium present in barley than of the same amount of selenium in meatmeal. Among the grains, wheat selenium had the highest bioavailability for the chick, barley selenium showed a distinctly lower value, and oats selenium was the poorest source. For products of animal origin the bioavailability of selenium in fishmeal was twice as high as that in meatmeal irrespective of the bioassay used.

Table 1. Bioavailability of selenium (%) in wheat, barley, oats, fishmeal. meatmeal and selenomethionine compared to that of sodium delenite (100%)

Source of Se	Bioassy							Reference
	Induction of GSH-Px		Prevention of exudative diathesis	Se concentration				
	Plasma	Whole blood		Plasma	Whole blood	Liver	Cardiac muscle	
Wheat	79	—	100	—	—	—	108	a*
	83	—	—	109	—	140	—	c
	90	—	—	107	—	151	—	c*
	—	80	—	—	123	—	—	f
Barley	71	—	85	—	—	—	87	a*
	77	—	—	151	—	82	—	d
	80	—	—	102	—	90	—	d*
	—	83	—	—	104	—	—	e
Oats	33	—	41	—	—	—	60	b*
	37	—	—	90	—	67	—	d
	62	—	—	107	—	98	—	d*
	—	46	—	—	99	—	—	e
Fishmeal	66	—	80	—	—	—	100	a*
	61	—	—	85	—	113	—	c
	73	—	—	86	—	119	—	c*
	—	56	—	—	107	—	—	f
Meatmeal	21	—	30	—	—	—	42	b*
	20	—	—	40	—	26	—	d
	26	—	—	47	—	31	—	d*
	—	21	—	—	69	—	—	e
Selenomethionine	77	—	77	—	—	—	114	b

a Hassan, S. 1986
b Hassan, S. 1987b
c Hassan, S., R.V.J. Hakkarainen & P.O. Lindberg 1987a
d Hassan, S., R.V.J. Hakkarainen & P.O. Lindberg 1987b
e Hassan, S. R.V.J. Hakkarainen, P.O. Lindberg & S. Sankari 1990
f Hassan, S., J. Hakkarainen, P. Lindberg & S. Sankari 1993
* Selenium sources were ethanol extracted

With respect to the profusion of contradictory information in the literature about selenium bioavailability, no single factor in the different studies may be designated as being the cause of the discrepancies. Probably, the major problem is that of methodology. A failure completely to remove vitamin E from natural selenium sources when using the prevention of disease as a bioassay may be one contributory factor. A lack of analytical precision and accuracy in the determination of low levels of selenium and vitamin E, both in diets and in animal tissues, which was particular problem during the earlier decades of bioavailability

research, in addition to continuous difficulties in GSH-Px analysis, may partially explain the contradictory selenium bioavailability figures reported. Besides these methodological difficulties, the choice of tissue to reflect GSH-Px status is also of importance. As observed by Lawrence & Burk (1978), the distribution of GSH-Px activity in the tissues differs with species; therefore, the tissue response with respect to this enzyme may also be species specific.

Studies on the bioavailability of selenium have preferentially been conducted with chicks, rats, lambs and turkeys, and on occasion, with pigs and calves. Within certain limitations, the observations in one species for a specific source may be applicable to another species. A review of the available literature generally shows no discernible trends for a better utilization of selenium from (a) specific source(s) by a particular species.

Selenium may be retained in animal tissues in its inactive form, or in forms which depend on the original chemical form in the specific source, feed or food, or on interactions with other minerals. In addition, if given in excess of the nutritional requirement, it will be retained in a storage form, substituting for sulphur in sulphur containing amino acids. The concentration of selenium in the target organ may be of significance in some selenium deficiency diseases. However, it must be clearly emphasized that the amount of selenium deposited in tissues cannot be generally considered as a criterion for biological activity. On the other hand, it cannot be denied that tissue selenium can have a very significant functional purpose as a storage depot for selenium needed for the synthesis of GSH-Px and other possible and essential selenoproteins, if the dietary intake of selenium becomes insufficient.

However, most of the contradictory information in the literature originates from the fact that often the concepts are mixed when drawing conclusions. In other words, there is a basic confusion of ideas about the bioavailability of selenium. Bioavailability should not only include the absorption of a trace element from the gastrointestinal tract and its excretion, it should, above all include its biopotency (Levander 1983). For my part, it is easy to understand that both the preventive approach and functional assay actually measure and estimate the biological activity of selenium, its biopotency. However, it is not equally easy to understand the usage of the word "bioavailability" in the connection with the tissue residue level approach. Here it is rather a question of the "biostorage" effect of dietary selenium, which of course may be of beneficial use in the body at a later time, when the storage form of selenium reaches the degradative metabolic pathways. This is especially relevant and significant for an animal when the optimal dietary intake for some reason happens to be restricted.

In conclusion, it is necessary to emphasize that the concept of biopotency is actually also included in the definition of bioavailability as proposed by Fox et al. (1981): "a quantitative measure of the utilization of a nutrient under specific conditions to support the organism's normal structure and physiological processes".

ORGANIC CONTRA INORGANIC SELENIUM

The different selenocompounds in feed- and food-stuffs, including supplemented sodium selenite, are nearly all available for the synthesis of essential, functional selenoproteins such

as glutathione peroxidase and iodothyronine deiodinase. However, as previously mentioned, the selenium in these compounds has different degrees of biological availability (Moksnes & Norheim 1986; Whanger 1986; Deagen et al. 1987; Waschulewski & Sunde 1988; Butler et al. 1989, 1990).

Selenomethionine is primarily used for the synthesis of tissue proteins, especially if the animal is deficient in protein or methionine. Supplementation with "ordinary" methionine promotes the metabolism of selenomethionine towards forms which are usable for GSH-Px synthesis, while at the same time reducing its incorporation into "ordinary" body proteins. Methionine deficiency does not have a similar effect on selenium bioavailability in sodium selenite. Instead, it may reduce the elimination of selenide from the organism and thus affect the toxicity of the inorganic selenium (Salbe & Levander 1990).

It has long been known that selenite selenium can be directly used in the organism for the synthesis of GSH-Px. On the other hand, the bioavailability of organic selenium for GSH-Px synthesis depends upon its ability first to be metabolized to selenides and selenocysteine. A vitamin B_6 deficiency, that is, pyridoxine, has a greater effect on the metabolism of selenomethionine than on that of selenocysteine or selenite (Yasumoto et al 1979; Beilstein & Whanger 1989).

Thus, the various organic selenium compounds are not necessarily metabolized to common intermediates. In this connection, it is interesting to note that selenium yeast contains about 50% selenomethionine, and the amount of inorganic selenium as selenite is estimated to be of the order of a few per cent (Korhola et al. 1986). The remaining selenium in the yeast is found as selenoglutathione, selenodiglutathione, selenocysteine and as other, unidentified, selenium-containing compounds.

In the discussion about the advantages of organic selenium in comparison with those of inorganic selenite, a few investigations are often cited where the conclusions have been that inorganic selenium is more likely to have functioned as a pro- than as an antioxidant (Dougherty & Hoekstra 1982; Csallany & Menken 1986; Levander & Burk 1986). The data presented in these papers are not very convincing. However, it is possible that even sodium selenite during certain exceptional experimental conditions can, just like almost all antioxidative substances, function as a pro-oxidant. On the other hand, under practical conditions the risk of this occurring is probably very small, especially when the successful usage of sodium selenite for prophylactic and therapeutic purposes in veterinary medicine to eliminate selenium deficiency diseases among our domestic and experimental animals is considered.

When sodium selenite is used for ruminants, attention should be instead concentrated on the effect of reducing substances, for example, vitamin C, which is present in the feed, and on the reducing effect of the microflora in the rumen. However, cattle apparently display rather peculiar characteristics with respect to the metabolism of dietary organic and inorganic selenium. Pehrson et al. (1989) found that yeast selenium and selenomethionine were about twice as active as selenite for increasing GSH-Px activity in the erythrocytes of selenium-deficient young cattle.

The general idea that selenium from organic sources is always retained in the animal body at higher levels than is selenium from inorganic sources, is not always true under all circumstances; it would be true if all the organic selenium was present as selenomethionine, but not if it was selenocysteine. In fact, selenocysteine is metabolized more like selenite

than selenomethionine (Sunde & Hoekstra 1980; Beilstein & Whanger 1986; Deagen et al. 1987). Thus, it is incorrect to assume that all organic selenium is metabolized similarly. The chemical form and composition of selenium in feed- or food-stuffs must be known in order to assess the metabolic pathways.

The risk of tissue accumulation during a long-term supplementation using organic selenium must be taken very seriously. The corresponding risk is considerably lower for inorganic selenium compounds since they can easily be excreted in the urine and through expiration.

There is a particular problem with the selenium content of mammalian milk, including human milk (Kumpulainen et al. 1985; Aspila 1991). Aspila (1991) concludes that cows must be fed either 0.7 ppm inorganic or 0.1 ppm organic selenium to obtain a milk selenium level optimum for human consumption of about 20 μg/l. This is another illustration of the great differences in the metabolic pathways between organic and inorganic selenium compounds.

"CLINICAL AND SUBCLINICAL" TOXICITY OF SELENIUM

At suitable dosages, selenium is a highly essential substance. However, much too often it is forgotten that it is, and will always be, a *trace* element, and one that is also toxic at higher levels. Regulations set by the members of the European Common Market allow a maximum content of 0.5 ppm in commercial feeds for all kinds of domestic animals. This covers the normal basal requirements and also the more diffuse needs, such as those for optimal growth and immunological preparedness.

The limit for chronic toxicity is about 3-5 ppm, and not at 10 ppm as previously believed. However, more recent investigation results recommend even greater caution. Negative physiological effects, among other things, a decreased resistance to disease, poorer growth and other signs of toxicity have been noted in the literature even at levels around 1 ppm (Witting & Horwitt 1964; Spallholz et al. 1973a, b; Larsen & Tollersrud 1981; Moksnes 1986; Larsen et al. 1988). It is also suggested that organic selenium compounds, e.g., selenomethionine, probably because of its enhanced bioavailability, may also have enhanced immunological cytotoxicity. According to Thorlacius-Ussing (1990), selenium occurs in rather high concentrations in the anterior pituitary of rats and humans and an overdosage of selenite results in a long-lasting toxic effect on the growth performance of young rats. Therefore, that particular care must be taken with respect to the oversupplementation of selenium to growing and pregnant mammals, needs no further justification.

The "subclinical" area of selenium toxicity has, however peculiarly, not been of interest to many researchers. On the other hand, a sufficiently sensitive, easily performed and highly specific method for measuring and evaluating the effect of a possible "subclinical" overdose of selenium is not known at the present. The only indication available is the previously mentioned negative effect on the immune system, which is a laborious method to use.

The respect for selenium as a potentially toxic element must not be lost. Selenium is a trace element baring the two faces of Janus; in reasonable amounts it is an essential

element. Its life-supporting and protective role can, however, almost imperceptibly and diffusely slide over to the dark toxic side, if one is not careful with the supplementation levels. Deliberately to increase the concentration of selenium in feedstuffs in excess of the animals' requirements, possibly at the expense of their well-being, is from the viewpoint of veterinary medicine, unethical.

On the basis of the above discussion with respect to the metabolism and toxicity of the different selenocompounds, it is also apparent that one should as quickly as possible consider carefully the question: "Is the intake of organic and inorganic dietary selenium of equal value for fulfilling animal requirements and at the evaluation of possible subtoxic and toxic risks for animal and man?"

CONCLUSIONS

In studies and investigations of the bioavailability of selenium test methods based upon the prevention of deficiency diseases or the measurement of the enzyme glutathione peroxidase have a greater significance for the measurement of real biological activity than does measuring selenium retention in the tissues. The latter method does not illustrate the actual biopotency; it rather illustrates a "biostorage" of selenium. However, properly used, the selenium retention method is a valuable complement to the other methods. In general, it can be stated that information obtained about the biopotency of selenium is more reliable and comprehensive if it has been determined using several different parameters and methods in parallel. Under these conditions, the retention test is also a valuable parameter, especially with respect to optimal human nutrition.

While the concept of selenium bioavilability is useful in facilitating the evaluation of the adequacy of specific diets and patterns of feed or food intake, it is very important indeed to recall that these estimates of bioavailability are always experimentally derived values. As such, these estimates must be considered in the context of the biological responses and biochemical conditions on which they are based. Equally important, it should be noted that the evaluations of selenium bioavailability are at best only that and just provide average figures which are intimately bound to the experimental limitations for the results obtained.

The clearly defined possibility of a wide variation in the bioavailability of selenium requires that the form of selenium compounds in feed- or food-stuffs should be established, in addition to the total selenium content.

It must also be empasized that accurate and reliable information about the bioavailability of selenium is of vital importance, if the optimal intake of selenium is a goal, both to avoid economic losses in animal production and to guarantee the optimal health and production of domestic animals. With respect to human nutrition, accurate information about the optimal intake of this trace element in domestic animals is equally important in order to satisfy the consumer's well-justified demands for nutritionally adequate food. However, the last-mentioned goal should not be achieved at the cost of animal health and welfare. Avoiding this implies the necessity for more basic research on the biological activity and metabolism of selenium.

With respect to domestic animals, selenium and vitamin E are the most critical and

first limiting feeding-dependent nutrients among the antioxidants. However, the chances that subsequent research will find functions of selenium of the same decisive importance as those already known are not too great. At the same time, the present knowledge in the area of disease caused by vitamin E and selenium deficiencies in domestic animals indicates that the vitamin E deficiency rather than the selenium deficiency could be the precipitating factor.

A very important task for future research is to evaluate as soon as possible both the nutritional physiology and the toxic effects of inorganic and of organic selenium, and their comparative importance for the selenium status and well-being of mammals. Actually, it might be that both these forms of selenium are necessary for supplementation to cover all the animal requirements, even those as yet unknown nutritional physiological needs and to guarantee nutritious animal products for human consumption! While this is being investigated, the well-documented fact that selenium is toxic at even moderate overdoses should not be ignored; the difference between optimal and toxic dietary levels appears to be shrinking.

"Alle Dinge sind Gift und nichts ist ohne Gift: allein die Dosis macht, dass ein Ding nicht Gift ist." These words are just as true today, especially with respect to selenium and its bioavailability, as when they were first spoken by Paracelsus (1493-1541) nearly 500 years ago.

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4 Acute and chronic selenium toxicity

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Because deficiences of vitamin E and selenium have been reported to contribute to various veterinary disorders, some livestock owners routinely administer these compounds to their animals, sometimes by various routes and in excess. The potential danger of selenium overdosage should not be underestimated. Acute selenium toxicosis should be considered in cases where fatigue symptoms are followed by sudden death; especially when pulmonary oedema, tissue haemorrhage, tissue oedema or methaemoglobinaemia is evident. Chronic selenosis typically includes effects on keratinized tissues, such as loss of hair, lesions on nails, claws, hoofs and skin. The sources of excess selenium and mechanisms of toxicity are discussed.

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Biologists first focused their attention on selenium (Se) in the 1930s when the element was identified as the toxic principle in plants that induced hair loss, lameness, hoof sloughing, and death in grazing livestock in certain areas of the American plains; especially in South Dakota and Wyoming (Franke 1934). This symptom, affecting keratinized parts of the body was called "alkali disease". This was because the occurrence of the disease was associated with alkaline soils on which the animals grazed. The subacute disease with neural symptoms was termed "blind staggers".

Selenium was considered solely from the toxicological point of view until Schwarz & Foltz (1957) described selenium as an essential trace element that is required in minute amounts. In 1973 the biological function of selenium as the catalytic component of the enzyme glutathione peroxidase became evident (Rotruck et al. 1973). This selenoenzyme has a central role in the antioxidant armamentum of the body. Selenium deficiency was seen to contribute to various disorders. Some areas of the United States (northeast, northwest) and other parts of the world, especially Scandinavia and New Zealand, have been identified as places where plants or feeds do not contain sufficient Se to meet animal requirements. This has led to use of selenium supplements - sometimes in excess. There is a potential danger of overdosing as the "therapeutic index" of selenium is one of the lowest among elements (Fig. 1). Many countries approved Se supplementation of livestock feeds at 0.1-0.3 ppm. The Food and Nutrition Board of the National Academy of Sciences (1980) suggested 5 mg/Se/kg diet as the critical level between toxic and nontoxic feeds. Overdosing might occur as a result of premix manufacturing errors or concurrent supplementation by several routes.

ECOTOXICOLOGICAL ASPECTS

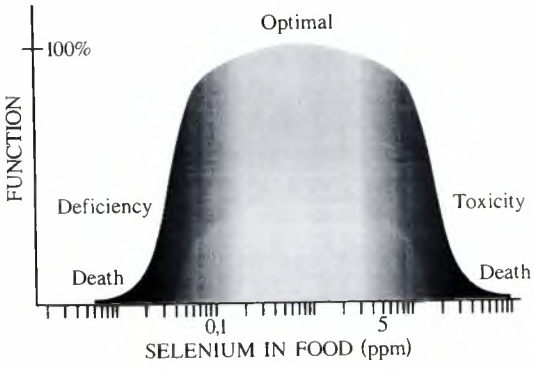

Fig. 1. Optimal range of selenium uptake

Soil mapping efforts have identified Se-deficient areas and Se-rich areas (Allaway 1972; Nriagu 1989). The concentration of total Se in most soils lies between 0.1 and 2 mg Se/kg. In seleniferous areas of the world, the total concentration of Se may be as high as 1200 mg/kg. In addition to the western parts of the American plains, localized seleniferous areas have been identified in Australia, China, Ireland, Israel, Russia, South Africa and Venezuela. Sedimentary rocks such as shales are usually high in selenium. Large areas of the semiarid western United States overlie seleniferous geological materials of Cretaceous origin. Water and plants from these areas are also likely to be seleniferous. Soils of the western plains of the USA, tend to have high Se levels, ranging from 2 to 10 mg Se/kg. The chemical forms of Se present in soils and sediments and their solubility are closely related to the oxidation-reduction potential and pH of the soil. Inorganic Se exists in the selenate or selenite form in aerated, alkaline soils. In poorly aerated acidic soils, inorganic Se exists as selenide or elemental selenium. In well aerated alkaline soils, selenium becomes leached to draining waters as selenate.

SOIL-PLANT-ANIMAL RELATIONSHIP

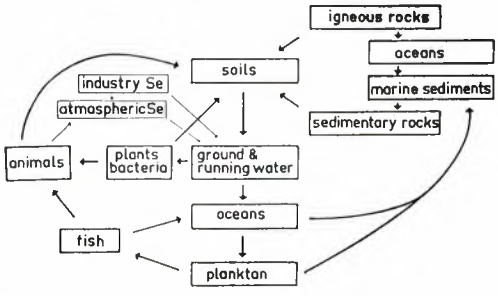

Fig. 2. Global cycling of selenium

Animals receive their selenium from plants. Selenium concentrations in animal tissues clearly tend to reflect dietary levels. A direct relationship is known to exist between the selenium content of forage plants and tissues of herbivorous animals feeding on them. Selenium is one of the few elements that can be absorbed in toxic concentrations by plants from soils. The bioavailability of soil Se for plants is: selenate >selenite >>elemental Se = selenide (Mayland & James 1988). All livestock and humans are known to be susceptible to Se poisoning. However, poisoning is most likely to occur in animals grazing seleniferous forage. Poisoning may occur in all animal species due to inclusion of seleniferous grain in the diet, or excessive selenium supplementation.

Plants have been divided into three groups on the basis of their tendency to accumulate Se when grown on high-Se soils:

I. The selenium accumulator or indicator plants grow well on seleniferous soils, and their presence is indicative of seleniferous soils. Plants in this group include several species of *Astragalus, Machaeranthera, Haplopappus*, and *Stanleya*. These plants may require selenium. On the other hand, these plants have developed systems to metabolize selenium to harmless compounds. Formation of Se-methylselenocysteine and selenocystathione is typical of accumulator plants (Shrift 1973). Grazing animals tend to avoid these plants because they give off an offensive odour and are unpalatable. If eaten by animals when there is a lack of more suitable forage, toxicity may occur. The toxicity of Se-containing plants cannot be evaluated solely on their Se-content basis. Many Se-accumulating plants contain additional toxins, including swainosine and miserotoxin (Ogden et al. 1988).

II. The intermediate group may accumulate 50-100 mg Se/kg.

III. Many plants that can grow on seleniferous soils usually accumulate only low levels of Se, but may occasionally contain up to 50 mg Se/kg. These plants, which can be called "passive accumulators", include common grains, grasses, white clover, etc. This group is the most important one as it contains palatable species that are likely to be grazed. In these plants most of the Se occurs in water-soluble forms such as selenate and seleno-amino acids.

Selenium toxicity in grazing animals was classified by Rosenfeld & Beath (1964) as follows: (1) acute intoxication and (2) chronic intoxication including the syndromes "blind staggers" and "alkali disease".

"Blind staggers" represents subacute selenosis with neurological signs, e.g. blindness, disorientation, ataxia. The syndrome occurs when cattle and sheep eat a sufficient quantity of seleniferous plants like *Astragalus* to cause severe toxic signs. Death may follow in a few hours. The neurological disorder has been attributed to the high levels of inorganic Se in these plants, but other toxins in the plants may contribute to this neurological disorder. "Alkali disease", which is characterized by loss of hair, inappetence, and elongated, cracking hoofs, occurs when animals, over a long period of time, graze Group III forages containing organic Se in moderate amounts (Mayland & James 1988). Selenium toxicity from plant sources cannot be compared directly with toxicity from inorganic selenium. Bacteria and plants convert inorganic selenium to organic forms (seleno-amino acids) more easily than animals do. Therefore, plant selenium is mainly in the form of seleno-amino acids. Selenomethionine and selenocysteine are less toxic than selenate or selenite in acute toxicity studies. This is probably because seleno-amino acids and inorganic selenium enter different metabolizing pools. On the other hand, organic forms of selenium tend to be more toxic in chronic toxicity tests. This reflects bioaccumulation due to incorporation of selenium into proteins.

AQUATIC ECOSYSTEMS

Bioaccumulation of Se occurs in aquatic food chains. This is principally attributable to selenium uptake by plankton (Sandholm et al. 1973). The accumulation should actually be called biomagnification. Selenium concentrations in fish (on whole body basis) are about 2-6 times those in producers (i.e. phytoplankton, algae) and sometimes 2000 times the concentration in water (Ohlendorf 1989). Bacteria, plankton and algae may take up selenium and convert it to organic forms. Soil and water bacteria tend to reduce selenate (Maiers et al. 1988). One of the reduction products, elemental selenium, is insoluble. Therefore, indigenous bacteria and algae have a significant role in the biochemical cycling of selenium. As with plants, some species of bacteria and plankton actively concentrate selenium but it is toxic to others.

Heavy metals (Hg, Cd) and selenium tend to move together in aquatic ecosystems. The toxicity of selenium and heavy metals is decreased when administered together to fish, birds and mammals. In aquatic mammals, such as seals, there is a close parallel between heavy metal and the selenium content of the organs (Koeman et al. 1973).

KESTERSON EXPERIENCE

In the San Joachin Valley, central California, the subsurface drainwater and agricultural return-flows contain appreciable amounts of selenium. The water in the Kesterson Reservoir has become toxic to fish and aquatic birds. The Kesterson Reservoir was essentially a waste-water sump that received irrigation drainage waters from the area. Bioaccumulation in natural waters seems to occur via the water-plankton-fish-bird ecosystem. It has been found that 10 μg Se/l in the water of a natural ecosystem can adversely affect bluegills, *Lepomis macrochirus*, (Hermanutz et al. 1992). Elevated concentrations of Se have already led to public health advice concerning the consumption of fish (Fan et al. 1988; Saiki 1990). The high selenium intake in aquatic birds in the National Wildlife Refuge Area at the Kesterson Reservoir has been linked with deaths and deformities in aquatic birds (Ohlendorf 1989). Certainly, events at the Kesterson Reservoir stimulated great interest in Se as a potential environmental hazard. This has led to pressure on authorities to take a more careful look at current feed additive regulations and the use of selenium in fertilizers.

METABOLIC TRANSFORMATION

The metabolic transformations of selenium depend on the species of plant or animal, the chemical form supplied to the organism and the amount of selenium supplied. Plants readily convert soluble inorganic forms of selenium to various organic selenium compounds, such as selenomethionine, selenocysteine, Se-methylselenocysteine and selenocystathione (Shamberger 1983).

ORGANIC FORMS OF SELENIUM (SELENO-AMINO ACIDS)

Seleno-amino acids enter the amino acid pool and behave much like sulphuric amino acids. Seleno-amino acids become incorporated in animal protein during protein synthesis (Fig. 3). This means that seleno-amino acids become slowly incorporated in tissue protein, such as muscle. In eggs, organic selenium accumulates in egg white whereas inorganic forms are mostly found in the yolk. In tissues where the protein synthesis is rapid, selenomethionine accumulates rapidly. This is the basis of why intravenously administered ^{75}Se-methionine works so well in scintigraphy of the pancreas and analysis of its exocrine secretion. Seleno-amino acids compete with sulphur-amino acids methionine and cysteine during protein synthesis. Selenomethionine can also be catabolised to selenide and then follow the methylation pathway typical to selenides (Fig. 3).

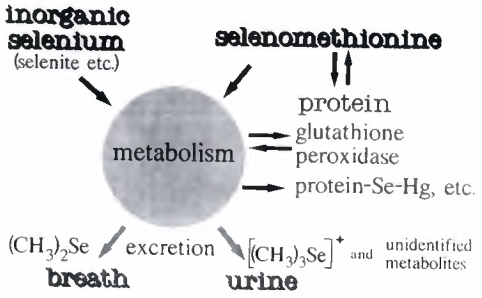

Fig. 3. Selenium metabolism in animals

INORGANIC SELENIUM

Animals differentiate between inorganic selenium and sulphur compounds, and not between the organic forms of selenium and sulphur. Inorganic selenium occurs with various oxidation states (Table 1).

Table 1. Examples of inorganic selenium compounds including the reductive pathway

Compound	Formula	Valency of Se		
Selenate	SeO_4^{--}	+ 6		
Selenite	SeO_3^{--}	+ 4	⇓	REDUCTIVE
Selenium (metallic)	Se^0	0		PATHWAY
Selenide	Se^{--}	− 2		

Selenium compounds follow reductive pathways in animal tissues but analogous sulphur compounds follow oxidative pathways. Sulphates and sulphate conjugates are major excretory products of sulphuric compounds. Selenium from inorganic sources (selenate, selenite), upon entering the circulatory system, accumulates within the first minute in the red blood cells. The selenium is expelled from the red blood cells and is taken up again by the liver, kidney, spleen brain and testes (Sandholm 1973a). When ^{75}Se-selenite was injected i.v. in mice, it immediately entered the red blood cells but was expelled within the

next few minutes (Sandholm 1973a). The radioactive selenium was then taken up by the liver, kidney and other organs (Gunn et al. 1967; Sandholm 1973a). When the dose was increased serially, the intracellular reduced glutathione (GSH) dropped in parallel. Apparently, selenite-Se reacts with intracellular GSH to form a selenodiglutathione (GS-Se-SG) trisulphide via the Painter's reaction; 4 RSH + H_2SeO_3 → RS-Se-SR + RSSR + 3 H_2O (Sandholm & Sipponen 1973). Either the selenotrisulphide is actively expelled from the cells or the expulsion is as selenide.

The effect of selenium on erythrocytes was mainly due to the i.v. route of administration. In this case, erythrocytes functioned as the primary glutathione-rich acceptors. In peroral dosing the enterocyte and the liver would be the first targets and in inhalational exposure the alveolar epithelium, respectively (Anundi et al. 1984b). The erythrocyte first-pass detected *in vivo* and the effect of selenite on reduced glutathione content and methaemoglobin formation can be reproduced on fresh anticoagulated whole blood *in vitro* (Sandholm 1973b). When the glutathione was depleted chemically from the erythrocytes, selenium did not move out from the cells and the inward flux became delayed (Sandholm 1973b).

Methylated selenides are the major excretory product of selenium in animals (Ganther & Hsieh 1974). At high levels of exposure, the trimethylselenonium ion is the principal excretory product in the urine (Fig. 3). In addition, urine often contains unidentified selenium metabolites which might be analogous with mercapturic acid-type metabolites. Exhaled dimethylselenide and dimethyldiselenide are volatile. When occurring in the breath, they give off a garlic odour. Volatile methylated selenides are formed only when animals are challenged with toxic doses of selenium. The fact that dimethyl selenide is less toxic than an equal amount of selenite illustrates the importance of methylation reactions in detoxifying excess selenium. The efficacy of methylation and excretion by exhalation is species-dependent. Rodents are generally good methylators. S-adenosylmethionine functions as the methyl donor for the methylation reaction.

Selenium from inorganic sources does not accumulate in protein-rich tissues like muscle, as do seleno-amino acids. This is an important aspect to consider when analysing potential risk for human beings consuming tissues from selenium-supplemented animals. On the other hand, inorganic selenium might form complexes with sulphur-rich proteins via the selenotrisulphide (RS-Se-SR) complexing principle. After acute exposure to inorganic selenium, the highest concentrations of selenium are found in metabolizing and excretory tissues, the liver and kidney. The minimum lethal dose of inorganic selenium (administered orally as selenite) is about 3 mg/kg for cattle and horses (Miller & Williams 1940; Rosenfeld & Beath 1964). A single subcutaneous dose of 2 mg selenium/kg b.w. (as selenite) was lethal to swine in four hours (Orstadius 1960).

ABSORPTION AND EXCRETION OF SELENIUM

Soluble selenium compounds are effectively absorbed from the gastrointestinal tract in monogastric animal species. Absorption efficacy figures ranging from 60% to 95% have been given for selenite, selenate, selenomethionine and selenocysteine. There appears to be no homeostatic control of the absorption of selenite. Selenomethionine competes with

methionine at the absorption site. Urine is the primary route of Se excretion by monogastric animals, regardless of whether Se is given p.o. or injected. Kidneys play an important role in the homeostasis of selenium. At low selenium intakes the body conserves selenium. Above a dietary selenium intake threshold, at about 0.07 mg selenite-Se/kg b.w., the urinary selenium excretion is directly related to dietary intake. The trimethylselenonium ion is a major urinary Se metabolite at high selenium intakes (Fig. 3).

In ruminants, the absorption and excretory pathway of inorganic selenium is dependent on the age of the animal as well as on the route of administration. Young ruminants resemble monogastric animals. Young preruminant calves and lambs excrete about 70% of the orally ingested Se in the urine. In adult ruminants, the rumen microflora has a profound effect. The rumen is a highly reducing environment. Much of the orally administered selenium is reduced to insoluble elemental selenium precipitate (Se^0) and appears in the faeces.

ACUTE TOXICITY

The toxic dose depends on chemical form, route of administration, duration of exposure, and animal species. The oral LD_{50} dose of Se as sodium selenite in non-ruminants is about 6 mg/Se/kg b.w. (single dose) and the parenteral LD_{50} value is about 2 mg Se/kg b.w. (Combs & Combs 1986). Acute selenium toxicosis induced by injectable selenium compounds has been reported in lambs, calves, pigs, dogs and laboratory rodents (Orstadius 1960; Caravaggi et al. 1970; MacDonald et al. 1981; Janke, 1989).

Affected animals stand with head lowered and ears drooping. Their temperature is slightly elevated and the pulse is rapid and weak (Rosenfeld & Beath 1964). Urine excretion is increased. The pupils are dilated. The mucous membranes are pale and discoloured. Death, which is preceded by collapse and prostration, is caused by respiratory failure. The main symptoms of acute sodium selenite intoxication are sedation, uncoordinated gait, followed by dyspnea and paralysis. The cause of death is apparently due to respiratory failure (Lin-Shiau et al. 1990). Intravenous dosing of sodium selenite (2 mg Se/kg b.w. i.v.) to pigs dramatically decreased the systemic blood pressure and increased cardiac output and heart frequency. Apparently sodium selenite induced a vasculogenic shock without primarily affecting cardiac performance. The only significant effect of dimethylselenide was increased cardiac output (Nebbia et al. 1991). The most characteristic sign of acute selenosis is "garlic breath" due to pulmonary excretion of volatile selenium metabolites, such as dimethyl selenide (Fig. 3).

When dosing mice i.v. with graded amounts of selenite, the lethal dose of selenite appeared to be the one which dropped the acid-soluble thiol content (principally reduced glutathione) of the liver and brain to zero. With this LD_{50} dose of selenium (2 mg/kg b.w. selenite-selenium into male mice, infused intravenously into the tail vein within 1 min), half of the mice collapsed but recovered within the next few minutes (Sandholm unpublished). The animals showed distinct methaemoglobinaemia (brown colouration of the blood). When pigs were administered i.v. with 2 mg Se/kg b.w. as either sodium selenite or dimethylselenide, a marked inhibition of subcellular succinic dehydrogenase and an increase in NADP-isocitric dehydrogenase and lactic dehydrogenase were noted. These changes

indicate a shift toward anaerobiosis. The cationic profile of plasma indicated alterations in cell membrane permeability and problems in maintaining cationic gradients across cell membranes (Nebbia et al. 1990). Isolated nerve-muscle preparations show neuromuscular blockade and tetanic spasms when treated with sodium selenite (Lin-Shiau et al. 1990).

Selenium is usually considered as an antioxidant because of its function in glutathione peroxidase. In acute toxicity, selenium clearly functions as a pro-oxidant where selenium itself is reduced at the expense of reduced glutathione. The toxicity of selenite probably comes from seleno-diglutathione trisulphide (GS-Se-SG) or factors related to depletion of intracellular GSH. This is evident as the metabolite, dimethylselenide, is less toxic than selenite. Moreover, selenite infused i.v. together with reduced glutathione (GSH) is more toxic than selenite alone. The lethality of selenite is increased when GSH is co-administered with selenite (Watanabe et al. 1988). This is probably due to selenotrisulphide formation in the extracellular fluid space. Seleno-diglutathione trisulphide might be the toxic intermediate of selenium. Selenite, when mixed with GSH, immediately forms the seleno-diglutathione-trisulphide(Gs-Se-SG) complex (Sandholm & Sipponen 1973). Methaemoglobinaemia is an indication of ongoing oxidative stress. Oxidative damage is probably the mechanism underlying the acute toxicity of selenium. The change in intracellular redox systems is characterized by a decrease in reduced glutathione (GSH) and NADPH. This occurs in both erythrocytes and hepatocytes (Sandholm 1973; Anundi et al. 1984). Too rapid a selenium replacement in Se and vitamin E-deficient animals might be dangerous because of the pro-oxidant nature of high levels of selenite. Selenite induced a large increase in ethane exhalation in rats, which had been fed a diet deficient in vitamin E and selenium (Dougherty & Hoekstra 1982). This was not seen in vitamin E- and Se-adequate controls. Ethane exhalation is a sensitive indicator of *in vivo* lipid peroxidation.

High concentrations of selenium can damage most vital organs. Virtually every organ is affected during toxicosis. Pathological findings include congestion and haemorrhage in the liver, kidney, lungs, omasum, pancreas, gall bladder, spleen and lymph nodes. Fatty metamorphosis and focal necrosis of the liver is typical. Symmetrical, focal areas of vacuolation and neuronal necrosis, especially in the ventral horns of the spinal cord, are typical of selenium toxicity in swine (Stowe & Herdt 1992).

THERAPY OF ACUTE SELENIUM INTOXICATION

No effective treatment is available for acute selenium poisoning, primarily because death occurs quickly before the disease can be diagnosed. From a theoretical point of view, intravenous infusion of sulph-hydryl compounds, such as reduced glutathione, acetylcysteine, BAL or dithiothreitol would be the drugs for emergency therapy. Acetylcysteine could be given by the peroral route. However, this theoretical emergency treatment of injecting i.v. glutathione (GSH) or other sulph-hydryl compounds, does not protect against selenite toxicity - in fact the toxicity is increased. GSH would be needed intracellularly and not in the plasma phase.

CHRONIC TOXICITY

Chronic toxicity is manifested by inanition and weight loss. "Alkali disease" is characterized by dullness, lack of vitality, emaciation, rough coat, loss of hair, hoof changes starting around the coronary band, and lameness. Anaemia is a common manifestation of selenosis in all species. Keratinized tissues appear to be the main target tissues in chronic Se toxicity. Reduced reproductive performance is the most significant warning signal for "alkali disease" in livestock (Olson 1978). Reproductive problems have been seen in experiments on mice, rats, dogs, swine and cattle and birds. Females receiving excess Se in their feed or water usually produce fewer and smaller offspring with a high mortality (Ohlendorf 1989). Serum Se concentrations might increase to about 1 mg/l (Stowe & Herdt 1992). In Scandinavia, the average serum selenium levels in animals and man are around 0.1 mg/l.

EMBRYOTOXIC AND TERATOGENIC EFFECTS

Malformations in aquatic birds at the Kesterson Wildlife Sanctuary alarmed biologists of possible teratogenic effects. Avian embryos are very sensitive to the toxic effects of Se. Hatchability of fertile eggs is considered the most sensitive measure of Se toxicity. The hatchability of chicken eggs is reduced when dietary levels are 6-9 mg/kg feed. Fertility is not affected, but Se causes high rates of embryo mortality and teratogenicity (developmental abnormalities) in birds. Deformities of embryos can be produced by injection of Se compounds into chicken eggs (Palmer et al. 1973). Congenital abnormalities observed in wild birds at the Kesterson Reservoir were multiple including anophthalmia, microphthalmia, abnormal beaks, amelia, micromelia, ectodactyly and hydrocephaly (Ohlendorf et al. 1988).

Selenium administered to pregnant hamsters during critical stages of embryogenesis was clearly embryotoxic. Selenite, selenate and selenomethionine all induced malformations, mainly encephaloceles. Foetal body weights were reduced in a dose-dependent manner. Assigning a specific teratogenic effect to selenium was confounded by maternal toxicity (Ferm et al. 1990). Selenosis has caused congenital malformations in sheep and horses.

A selenium-rich premix added to the rations of sows during the second half of gestation resulted in haemorrhagic claw lesions in newborn piglets (Mesink et al. 1990).

INTERACTIONS OF Se WITH HEAVY METALS AND ARSENIC

The metabolism of selenium is strongly influenced by concurrent exposure to other elements, particularly heavy metals and arsenic. Heavy metals influence bioavailability, uptake, transport, and physiological activity of selenium.

Selenium has been shown to offer protection against heavy metal toxicities (Hg, Cd, Ag, Pb, Zn) (Parizek et al. 1971; Cuvin & Furness 1988). A reverse situation is also true, where heavy metals (Ag, Hg, Cd, Cu) and arsenic decrease toxicity of selenium. These

interactions have been described in fish, birds and mammals. Selenium does not inhibit absorption or enhance excretion of heavy metals. Actually an accumulation of heavy metals in organs such as the gastro-intestinal wall and liver takes place as a result of simultaneous administration of selenium and heavy metals. The explanation is that selenide complexes with heavy metals yielding stable, unmetabolizable, non-toxic compounds (Suzuki 1988) (Fig. 2). Apparently endogenous glutathione (GSH) is required to reduce selenite to selenide to support complex formation. Selenium has been shown to protect against methylmercury intoxication (Simplicio & Leonzio 1989). The high lipophility of bis-methylmercury selenide alters the distribution and metabolism of methylmercury and thus its toxicity.

PREVENTION AND THERAPY OF CHRONIC SELENOSIS IN ANIMALS

The possibilities for preventing chronic selenium poisoning in animals are: (1) The soil can be treated so that selenium uptake by plants is reduced. A decrease in soil pH is essential. Fertilization with sulphate, arsenite or arsenate might help; (2) the diet can be modified by adding substances which antagonize or inhibit toxic effects of selenium within the body; and (3) the animals can be treated so that the absorption is reduced or excretion is increased.

Results from many experiments have shown that it is possible to suppress toxicity of excess selenium with heavy metals (Hg, Ag, Cd, Cu). Because of bioaccumulation, this is not a practical solution for prevention or therapy of selenium toxicity in production animals.

Arsenic protects against chronic selenium toxicity, and sodium arsenite and arsenate are equally effective. The mechanism seems to be due to increased biliary excretion of selenium (Levander 1986). Urinary loss of selenium can be enhanced by the administration of p-bromobenzene to animals, but because of the hepatotoxicity of bromobenzene this has practical limitations.

Several studies indicate that selenium toxicity may be dependent on previous selenium intake. This indicates adaptation to selenium. Hepatic glutathione (GSH, GSSG, both) increases during chronic selenium administration. The increase in GSH can be regarded as an adaptive change in an attempt to maintain intracellular reductive balance.

Variability in sensitivity to Se poisoning may also exist due to previous or simultaneous exposure to other dietary and chemical factors, such as heavy metals (Levander 1986; Parizek 1990). Dietary modifications, such as high protein and sulphate intakes and supplementing the diet with arsenic, mercury or copper, all have potential for alleviating selenium toxicity. Because of several interactions, accurate recommendations for maximum dietary intake cannot be given.

SELENIUM TOXICITY IN MAN

In recent years, selenium has attracted considerable attention in human health and disease. Recent "health food" supplement campaigns have increased the risk of over-exposure to selenium due to self-medication. Several cases of human selenium toxicity as a result of self-medication have been reported. The most common symptoms in these cases were nausea, vomiting, nail changes, hair loss, fatigue and irritability. Abdominal cramps,

diarrhoea and garlicky breath were reported in addition (Levander 1986).

Selenium was not previously regarded as a serious toxic threat to human public health. Problems of toxicity were attributed to skin and eye contact as well as inhalation of fumes or dusts of selenium compounds by industrial workers exposed to selenium. Selenium dioxide is the most common selenium hazard encountered in industry. Exposure to selenium used to be common in Cu smelting and Se rectifier plants. Typical signs of contact with selenium dioxide are painful burning of skin and eyes, contact dermatitis and allergy. Symptoms from inhalation of selenium compounds include irritation to mucous membranes of the upper respiratory tract with the symptoms of tearing and burning sensation in the eyes. Skin rashes and indigestion are other common symptoms. The monitoring of urinary selenium levels in industrial workers exposed to selenium was suggested by Glover & Chir (1967). Industrial workers over-exposed to selenium occasionally have garlicky breath due to dimethylselenide, the volatile metabolite of selenium (Fig. 3).

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5 Methods for the evaluation of selenium status

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Selenium (Se) concentration and glutathione peroxidase (GSH-Px) activity in blood components and tissues are used for monitoring the selenium status of domestic livestock. Selenium in blood and tissues correlates well with Se intake in several animal species, although the erythrocytes respond more slowly to Se supplementation than other tissues. Incorporation of organic Se to various body proteins increases tissue Se content more efficiently than corresponding levels of inorganic Se. The biological significance of the stored Se should be evaluated in respect of adequate Se status based on the measurement of Se content in blood and tissues. GSH-Px is considered to be a measure of biologically active Se. The use of GSH-Px as an indicator of Se status requires knowledge of the correlation to Se and the differences expected due to species, age, sample material and selenium source.

Key words: Glutathione peroxidase, selenium, selenium status.

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Selenium (Se) concentration in whole blood, plasma or various tissues is used as a direct indicator of Se status in humans and animals. Analytical methods for Se have improved during the last few decades so that now even low Se levels in foods and feeds, as well as in blood and tissues of Se deficient animals, can be accurately determined.

Since the discovery that glutathione peroxidase (GSH-Px, EC 1.11.9.1) is a selenoenzyme (Rotruck et al. 1973; Flohé et al. 1973) the close correlation between GSH-Px and Se in blood components of various animal species has been demonstrated (Noguchi et al. 1973; Chow & Tappel 1974; Oh et al. 1974; Wilson & Judson 1976; Caple et al. 1978; Hakkarainen et al. 1978). GSH-Px activity in blood or plasma has been further shown to respond to dietary Se level (Hafeman et al. 1974; Oh et al. 1974; Hakkarainen et al. 1978; Thompson et al. 1981). Based on these findings, GSH-Px activity became a measure of the biologically active Se in animals. However, the use of GSH-Px as an indicator of Se status requires that the correlation between GSH-Px activity and Se concentration in sample material is verified by the GSH-Px method used.

ASSESSING Se STATUS BY BLOOD AND TISSUE Se CONCENTRATION

Selenium concentration in serum or plasma correlates well with oral and parenteral administration, and responds quickly to changes in intake of inorganic Se in particular (Oh

et al. 1974; Chavez 1979; Hassan et al. 1988). GSH-Px and other Se containing proteins synthetized by the body constitute a minor part of the serum and plasma Se, while Se in erythrocytes is mainly incorporated into GSH-Px in various animal species (Beilstein & Whanger 1983). Whole blood Se responds more slowly than serum Se to changes in Se intake, as erythrocyte GSH-Px is formed during erythropoiesis (Hafeman et al. 1974). Both red blood cell Se and GSH-Px are regarded as long-term indicators of Se status in animals because of the relatively long life span of erythrocytes.

The response in blood and tissue Se concentration to organic Se intake clearly differs from that to inorganic Se. Organic Se, frequently in the form of selenomethionine, is metabolized similarly to methionine. Incorporation of selenoamino acids into various serum and tissue proteins increases their Se content more efficiently than corresponding levels of dietary inorganic Se (Osman & Latshaw 1976; Mahan & Moxon 1978; Borglund & Åkesson 1987; Moksnes & Norheim 1986; Deagen et al. 1987). The distribution of Se to various organs depends on the Se source. Selenium level in muscle tissue is markedly higher in animals fed with organic Se than with corresponding amounts of inorganic Se.

The Se content of tissues can be affected by the intake of a nutrient other than Se. The restorative effect of selenomethionine on tissue, hair and nail Se in the rat is enhanced at insufficient dietary methionine levels (Salbe & Levander 1990 a, b), which complicates the interpretation of these variables in assessing Se status of humans and animals at limited dietary methionine levels. The difference in tissue Se response to organic and inorganic dietary Se sources should be taken into account when Se status is determined to be adequate based on serum or plasma Se concentrations and various indicators of animal health.

Genetical variation in ability to absorb or retain Se was reported in Finnsheep (Sankari & Atroshi 1983) and swine (Stowe & Miller 1985) and it may exist in other species. The role of genetic hyposelenemia in respect of swine health is under investigation (Horvath et al. 1992), and Se concentration may in the future become a versatile indicator of performance in swine.

The serum and tissue Se concentrations reported in connection with suspected Se toxicity clearly exceed the expected values (Stowe & Herdt 1992). An indicator for milder Se excess is still lacking. Kidney Se concentration has been suggested for monitoring Se toxicity, because of early accumulation of Se in kidney tissue (Salbe & Levander 1990b). Urinary Se could be a sensitive indicator of Se toxicosis because the excess Se is primarily excreted via the kidneys. In rats and humans, the excretory metabolite, trimethylselenonium, is present in the urine but represents less than 1% of urinary Se (Sun et al. 1987; Zeisel et al. 1987). The compostition of Se compounds in the urine of domestic animals is not yet known.

ASSESSING Se STATUS BY GSH-Px

Nutritional Se status has been assessed on the basis of GSH-Px activity in plasma and blood cells, which correlates to the Se concentration of the tissues. The information obtained using GSH-Px has been critizized as being inferior to that obtained from measurements of tissue Se concentration. The differences in specific assay conditions, acceptor substrate (H_2O_2, organic hydroperoxides), and in expression of the enzyme activity, cause consider-

able variation in results between laboratories. In addition, the role of GSH-Px in plasma is not clear. It may merely spill over from other tissues in which it has reached saturated levels (Combs & Combs 1986). GSH-Px activity may also increase in plasma irrespective of Se status, as a result of enzyme leakage from GSH-Px rich cells, e.g. from liver cells or erythrocytes.

GSH-Px in animal blood, plasma and erythrocytes has been shown to indicate Se status in individuals with low or moderate Se intakes. With increasing Se intake, the plasma GSH-Px reaches a plateau which indicates that linear correlation with plasma Se content, or dietary Se, becomes less apparent (Hakkarainen et al. 1978; Sankari 1985).

The large variation in GSH-Px activity between species and tissues has given rise to questions about the use of the enzyme as a tool for assessing selenium status (Levander 1986). Observed genetical and age-dependent variation in GSH-Px activity in sheep and pigs further complicates the interpretation of results in these species (Atroshi et al. 1981; Jørgensen et al. 1977). When GSH-Px activity is used as an indicator of Se status, it should always be taken into account that the correlation between GSH-Px and Se is dependent on the sample material, the methods used, and the age and species of animal examined.

In contrast to most animal species studied, the human erythrocyte GSH-Px accounts for only about 10% of the Se contained in the cells. This suggests that erythrocyte GSH-Px activity is not a good measure of Se status in humans (Beilstein & Whanger 1983). Platelet GSH-Px in human blood has, however, been shown to respond well to Se intake (Alfthan 1990).

ASSESSING LOW LEVELS OF Se

GSH-Px is considered to represent the biologically active form of Se in the tissues, whereas total Se concentration includes the Se stored in the body. Application of the two methods of assessment described above, at very low or excessive Se intake, results in difficulties and inaccuracy. The plasma GSH-Px activity does not respond to Se intake or to tissue GSH-Px at very low levels of supplemental Se (Combs & Combs 1986). Maintainance of GSH-Px level is suggested to be of secondary importance compared with newly found selenoproteins at low dietary Se intake (Behne 1988). The distribution of Se in rat tissues follows a characteristic order at adequate Se intake, but the priority of the tissues changes with Se depletion, and the metabolized selenium is probably reutilized by target tissues (Behne 1988). Thus the Se content of these tissues, including testes and adrenals, is not always a function of Se intake.

At high Se intake levels the erythrocyte or whole blood GSH-Px is shown to further increase in cattle (Carlström 1979; Pehrsson et al. 1989) while the plasma GSH-Px response generally ceases in animals. Similarly, at high inorganic Se supplementation levels a plateau is reached in plasma or milk Se concentration (Maus et al. 1980). Organic Se, in turn, efficiently increases tissue Se concentration, but the biological significance of the stored Se is not clear. Both methods have restricted use in evaluating Se status at high levels of Se intake.

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6 Notes and comments on the determination of selenium in biological materials

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This short review presents aspects of procedures for determination of selenium (Se) in biological materials in order to facilitate the choice of an appropriate analytical method. Only the most frequently used sample preparation procedures and various analytical techniques are listed. Automated wet ashing using a mixture of $HNO_3/HClO_4$, and flow-injection hydride-generationatomic absorption spectrometry (FI-HG-AAS) for Se determination are discussed in detail. Automated wet ashing has been successfully used for more than 20 years in our laboratory. Since 1986, experience in method development and in comprehensive routine work has been achieved by using a home-made, automated and computerized FI-HG-AAS. Recent determination methods of Se have more or less comparable sensitivity and detection limits. Thus, the choice of method is mainly influenced by economics and practical/routine requirements. Available equipment, skilled staff, as well as type and quality of sample, length of sample series, expected Se concentrations, are all decisive factors in the choice of method.

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In recent years intensive analytical research has resulted in the development and improvement of fast and inexpensive analytical methods for determination of selenium (Se). This is a prerequisite for increased knowledge and understanding of the biological role of this essential element.

In materials of biological and environmental origin Se is often present in concentrations at trace and ultratrace levels (i.e. < 1 μg/g, sometimes even < 0.1 μg/g). This demands highly sensitive analytical techniques. Special care must be taken when sampling, storing and preparing the samples in order to avoid, among other things, contamination and/or loss of the analyte (Fishbein 1991).

Reviews, summarizing the problems and perspectives in Se analysis, are reported in Table 1.

In the present paper an overview of the most frequently used methods in Se determination is presented; advantages and shortcomings are discussed. Since 1986 the flow-injection hydride-generation atomic absorption technique (FI-HG-AAS) and automated wet ashing have been applied in the analysis of Se at our department, giving us experience

in development and routine use of a method of great capacity and suitable sensitivity, and one that is easy to automate (Galgan & Frank 1988). Special attention is given to this system.

Table 1. Selenium determination in biological and environmental materials. Review Articles

Reference	Contents
Bock, R. et al 1964	Decomposition, enrichment and separation as well as determination methods
Campbell, A.D. 1984	Biological materials and water
Campbell, A.D. 1992	Hydride-generation techniques in atomic spectroscopy HG-AS; ICP-AES
Combs, G.F., Jr. & S.B. Combs 1986	Determination methods
Fishbein, L. 1991	Determination methods
Hofsommer, H.-J. & H.J. Bielig 1981	Determination in foodstuffs
Raptis, S.E. et al. 1983	Comprehensive literature review: sampling, decomposition, separation, determination methods
Robberecht, H. & R. Van Grieken 1982	Environmental waters: sampling, storage, preconcentration steps, determination methods
Tölg, G. 1984	Biological materials: most powerful methods, power of detection, reliability and economy

SAMPLING AND STORAGE

Detailed information about sampling and storage of biological materials for Se analysis is available in the literature (Bock et al. 1964; Sansoni & Iyengar 1980; Raptis et al. 1983). The prerequisite for precise analytical results is representative and homogeneous samples of the material being studied. Container material and surface area per unit sample, as well as pH of aqueous solutions might be important in adsorption and desorption of Se. Cleaning of glassware and plastic containers with 10% warm HNO_3 and careful rinsing with deionized water in our laboratory have proved to be satisfactory.

Oven-drying at temperatures below 120°C or lyophilization does not influence the Se content. In urine, Se losses of up to 30% were observed when drying at 80°C (Iyengar & Sansoni 1980).

SAMPLE PREPARATION

Comprehensive studies concerning different decomposition procedures in the analysis of Se in biological and environmental materials are reviewed by several authors (Bock et al. 1964; Raptis et al. 1983; Hansson 1989; Pettersson 1990). Some aspects of the prcedures are listed below.

The main principles of decomposition methods are as follows:

- wet decomposition in open and closed systems
- dry decomposition by fusion and muffle furnace ashing
- combustion methods in oxygen at normal pressure and at increased pressure
- decomposition in oxygen plasmas

When choosing a particular method, different aspects and parameters have to be taken into consideration (see Table 2).

Table 2. Frequently used methods for decomposition of biological materials in determination of selenium

Decomposition	System	Heating	Automation	Reagent	Ashing time	Max. sample amount	Comments
Wet ashing	Open (ashing tube)	Thermostat	Yes	HNO_3	Long	Variable	Incomplete mineralization
		i.a. Al-block	Yes	$HNO_3/HClO_4$	"	"	Safety precautions
		- -		$HNO_3/H_2SO_4/HClO_4$	"	"	" " ?
		Microwave	Yes?	$HNO_3/Mg(NO_3)_2 \cdot 6H_2O$	"	"	High salt content
	Closed (pressure bomb)	Thermostat	?				
		direct heat		HNO_3	Short	Small	Incomplete mineralization
		microwave	?				
Dry ashing	Open (muffle furnace)	Direct heat	Yes?	$Mg(NO_3)_2$ + MgO	Long	Large	High salt content
Combustion	Open	Ignition	?	Oxygen	Short	Small	
	Closed	RF-gen.	?	Active oxygen	Long	Variable	

Ref. Bock et al. 1964; Raptis et al. 1983; Tölg 1984

Many analytical methods used in the determination of total Se content in biological (and environmental) materials assume the complete destruction of the organic constituents. However, acid-resistant organoselenium compounds such as selenomethionine, selenocysteine and trimethylselenonium ion demand agents with a high oxidation potential for complete destruction (Verlinden 1982). Furthermore, HNO_3 alone or mixed with H_2SO_4 is insufficient to decompose materials with a high fat content.

Selenium readily forms volatile species and can be lost during an uncontrolled decomposition, for example by charring. In many cases $HClO_4$ is required to retain Se in solution. Mixtures of $HNO_3/HClO_4$ or $HNO_3/HClO_3/HClO_4$ enable complete destruction of biological materials at temperatures < 250 °C without losing Se. But $HClO_4$ must be handled with great care and following strict regulations; special equipment is necessary and precautions

have to be taken (see below).

Losses in open systems are minimized by using reflux condensers or long-necked digestion tubes (Robberecht et al. 1982; Verlinden 1982).

The possibility of contamination by dust in laboratory air has to be taken into consideration when using open systems.

Automated devices for routine analysis, such as electrically heated blocks (Frank 1976, 1983, 1988) and microwave systems (Kuss 1992) which control digestion time and temperature, are commercially available.

Wet decomposition in closed systems, such as pressure bombs or microwave equipments, avoids losses arising from volatilization and contamination by laboratory air. A variety of closed systems perform decomposition at elevated temperatures and pressure. The equipment is particularly suited to treatment of small sample amounts. However, the automation might be troublesome.

Decomposition by fusion with MgO/Na_2CO_3 and dry ashing with $Mg(NO_3)_2/MgO$ as additives are useful alternatives. However, some contamination may occur.

For combustion in oxygen at normal pressure, the ratio of sample quantity to surface of the apparatus must be favourable. In oxygen bombs, under pressure, the risk of explosion and Se losses exists. Samples up to 1 g can be decomposed in oxygen plasmas (high-frequency discharge and low power). However, slow sample throughput is a limiting factor.

Many analytical methods demand Se in the oxidation state Se(IV). After decomposition with oxidizing agents, Se(VI) has to be reduced to Se(IV), preferentially with HCl. The efficiency depends on HCl concentration, reduction time, and temperature of the solution (Piwonka et al. 1985).

DETERMINATION METHODS

Comments on requirements and criteria of routine methods for Se determination in biological materials are given in Table 3. Detection limits for these methods and for other sensitive analytical methods are presented in Table 4.

The most frequently used techniques in routine analysis are fluorimetry and atomic absorption spectrometry, the latter in combination with electrothermal atomic absorption spectrometry or hydride generation. Increased use of instruments for plasma-atomic emission, in combination with hydride technique as well as with mass spectrometery, opened a new field of application for single and simultaneous multielemental determinations of hydride-forming elements. Other methods of analytical importance are chromatographic and electrochemical techniques, X-ray fluorescence, proton-induced X-ray emission, and neutron activation analysis. Recently, there has been increased interest in methods for speciation of different Se compounds in biological materials.

Five instrumental methods, inter alia those mentioned above, were compared under standardized conditions for technical and practical criteria, concerning detection limits, reproducibility, time and cost (Haygarth et al. 1991).

Table 3. Comparison of routine methods for selenium determination in biological materials

Method	Requirements				Sample amount	Auto-mation	Inter-ference	Comments
	Decomposition	Reduction Se(IV)	Time	Personnel				
Fluorimetry	Yes	Yes	*	Intensive	Variable	Yes	Yes	Fluorescence from DAN
AAS								
ET-AAS	Partial	No	Low	Moderate	Small	"	"	Matrix modif. backgr. corr.
HG-AAS								
Batch	Yes	Yes	*	Moderate	Variable	?	"	Interf. from
Contin. flow	"	"	Low	"	"	Yes	(Yes)	hydride-forming
Flow inject.	"	"	"	"	"	"	(")	& transition metals
HG-ICP-AES HG-DCP-AES	"	"	"	"	"	"	(")	Multielement determination
HG-ICP-MS	"	"	"	Skilled staff	"	"	Yes	High capital costs

* time consuming

Table 4. Detection limits for sensitive selenium determination methods

Method	Detection limit		Reference
Fluorimetry	0.5	ng/ml	Raptis et al. 1983
ET-AAS	3	"	Welz et al. 1992
	2	ng/g	Hocquellet & Condillier 1991
HG-AAS			
Batch	< 1.0 - 10	ng/ml	Tölg 1984
	0.02	"	Mayer et al. 1992
Contin. flow	0.01	"	Piwonka et al. 1985
" "	0.2	"	Norheim & Haugen 1986
Flow injection	0.07	"	Galgan & Frank 1988
" "	0.1	"	Negretti de Brätter et al. 1990
" "	0.1	"	Pettersson 1990
" "	0.04	"	Völlkopf et al. 1990
HG-ICP-AES	0.3 - 0.8	"	Schramel & Xu 1991
	0.3	"	Nölte 1991
HG-ICP-MS	1.3	ng/g	Buckley et al. 1992
	0.03 - 0.2	ng/ml	Völlkopf et al. 1990
HPLC-UV	0.3 - 0.7	"	Robberecht & Van Grieken 1982
GC-EC	2.2	"	Al-Attar & Nickless 1990
	0.002	"	Raptis et al. 1983
GC-MS	2.8	"	Ducros & Favier 1992
ASV, CSV	< 1	"	Adeloju et al. 1983
ED-XRF, PIXE	2 - 8	"	Robberecht et al. 1990
RNAA	0.01	"	Tölg 1984
	0.025	ng/g	Haygarth et al. 1991

Fluorimetry

In the fluorimetric method, Se(IV) reacts with an aromatic diamine, often diaminonaphthalene (DAN), to form piazselenol, which is extracted into an organic solvent and then the fluorescence of the compound is measured (Lindberg 1968). Advantages of the DAN procedure are its good sensitivity and its relatively low cost (Combs & Combs 1986). However, the method is time-consuming, operator intensive and needs qualified, trained staff (Haygarth et al. 1991). Using high-performance liquid chromatography (HPLC), automation is possible (Handelman et al. 1989). Care must be taken in order to achieve the complete decomposition of the sample, and the reduction to Se(IV), as well as the interfering fluorescence of DAN itself, which needs purification.

Atomic absorption spectrometry

Conventional flame atomic absorption spectrometry (FAAS) is often unsuitable for the determination of selenium because of the generally high limit of detection (0.05 to 3 ppm). Improved sensitivity can be achieved by using electrothermal atomic absorption spectrometry (ET-AAS) with a graphite furnace. Direct Se determination, for example in blood and water samples without prior decomposition, is an interesting application (Angerer & Schaller 1988). Because of interference from, inter alia, iron, a Zeeman-effect background correction is necessary. Use of high temperature (e.g. atomization higher than 2000°C) reduces interference caused by non-specific absorption of organic compounds and non-Se salts, but introduces the problem of Se volatility. This can be avoided by using salts - matrix modifiers - for thermal stabilization of Se during the charring stage.

A palladium nitrate-magnesium nitrate modifier allows an interference-free determination of Se in mineral waters with a high sulphate content (Welz et al. 1992). The same modifier was used to determine Se in nutritional supplements and selenized yeast after ashing in nitric acid (Larsen & Ekelund 1989).

The hydride-generation atomic absorption (HG-AAS) technique has been used for determination of hydride-forming elements since the 1970s. These elements form volatile hydrogen compounds in the presence of a suitable reducing agent in acid solution. The gaseous compounds are transferred from the sample solution to a nitrogen or argon gas phase, and swept into a heated cuvette for atomization and determination. The theoretical background, mechanisms, interactions and disturbances, as well as applications are surveyed in the literature (Welz 1985; Campbell 1992).

Selenium hydrogen (SeH_2) is usually produced by reducing Se(IV) with sodiumtetrahydroborate ($NaBH_4$) in hydrochloric acid solution. The gas is swept into a heated quartz cuvette for atomization.

The hydride generation can be carried out in batch- and in continuous-systems.

The batch system has been used for a relatively long time. It can handle large sample volumes; thus, low detection limits can be achieved (see Table 4), which is the main advantage of this technique. However, the technique is laborious, time-consuming, and not well suited to automation. In addition, the relatively long reaction time can cause serious disturbances from interfering elements.

The use of equipment with continuous flow systems, maintained by peristaltic pump, handling sample and reagents simultaneously, has been an important improvement. Commercial equipment was launched during the 1980s (Sturman 1985). Application of the

technique for selenium determination in biological materials is described by Norheim & Haugen (1986).

For determination of extremely low amounts of the analyte a special trapping technique was developed (Piwonka et al. 1985). The method is useful for determination of low concentrations of Se (ng/g and p/g levels). However, the technique is time-consuming for use in routine work.

In later developments flow injection (FI) systems were also adopted. In a continuously flowing carrier stream discrete sample volumes are injected and mixed with the reagent solution. The analyte-containing solution is transported to the detector and results in a reproducible transient analytical signal (Ruzicka & Hansen 1975).

The combination of flow-injection technique with hydride-generation AAS (FI-HG-AAS) is described in several studies (Chan 1985; Yamamoto et al. 1985; Wang & Fang 1986; Fang et al. 1986; Galgan & Frank 1988; Negretti de Brätter et al. 1990; Pettersson 1990; Welz & Schubert-Jacobs 1991; Chan & Sadana 1992). Different aspects regarding the advantages and disadvantages of the system will be discussed below.

Plasma atomic emission spectrometry

An improved detection limit is achieved in determination of Se with direct current or inductively coupled plasma atomic emission spectrometry (HG-DCP-AES or HG-ICP-AES) when hydride generation is applied. Using plasma techniques, hydride-forming elements can be determined simultaneously.

When an ICP spectrometer is coupled directly to a continuous hydride generator unit, the carrier gas flow rate is critical in maintaining a stable plasma. Chemical parameters which influence the signal intensity and stability are pH, reaction time, and interelemental interference (Nölte 1991; Schramel & Xu 1991). In multielement determination for generation of hydrides the compromising of reaction conditions is necessary.

The combination of HG-ICP-AES with mass spectrometry (MS) allows multielement determination of hydride-forming elements at ultratrace level in environmental samples (detection limits 0.5 -7 ng/l) (Stroh & Völlkopf 1993). Interference from Ar-Cl on mass 77 can be prevented by removing Cl from the plasma using a tubular gas liquid membrane separator (Haygarth et al. 1991). However, the capital costs of the method are high.

Chromatographic techniques

With chromatographic techniques, such as high-performance liquid chromatography (HPLC) in combination with ultraviolet (UV) detector and gas chromatography with electron capture (GC-EC) detector, very low Se concentrations can be determined. The detection limits are given in Table 4. Selenium quantification is based on the detection of the amount of piazselenol formed in the reaction of Se(IV) with an appropriate reagent. Piazselenols can be extracted into organic solvents and injected into a GC or separated by HPLC. Detection limits are in the nano- and picogram range (Robberecht & Van Grieken 1982; Tölg 1984; Johansson & Olin 1992).

Electrochemical methods

Selenium concentrations of less than 1 ng/ml can be determined also by electrochemical methods such as anodic (ASV) and cathodic (CSV) stripping voltametry (Adeloju et al.

1983) (Table 4). Sample decomposition must be carried out very carefully and prerequisites include 100% mineralization and a highly experienced staff.

X-ray fluorescence

X-ray fluorescence (XRF) and the proton-induced X-ray emission technique (PIXE) permits the simultaneous determination of several trace elements. Solid samples can be analysed. But for improved detection limits (Table 4) a pre-concentration step is often necessary (Robberecht et al. 1990).

Neutron activation analysis

In neutron activation analysis (NAA) the power of detection for Se determination can be improved by radiochemical separation (RNAA). RNAA is a well-proven and robust method, with low detection limits (Table 4) and high precision. Digestion of the sample is not necessary. The method is time-consuming and expensive for routine investigations, as was observed in a comparison of five instrumental methods for the analysis of total Se (Haygarth et al. 1991). Nevertheless, neutron activation analysis is valuable for the certification of standard reference materials and control of the accuracy of other analytical methods.

Speciation of different Se compounds

A combination of different chromatographic methods with various detection techniques is used in speciation of Se compounds, e.g. the combination of HPLC with ICP-AES (Brätter et al. 1988), HPLC with ultraviolet and HG-DCP-AES detection (Childress et al. 1992), ion exchange chromatography with fluorimetry (Hasunuma et al. 1993) and reversed-phase liquid chromatography with electrochemical detection (Killa & Rabenstein 1988). The speciation technique is the key to knowledge about biological forms of Se and to understanding its biological role in the organism as well as its nutritional availability.

EXPERIENCE OF DECOMPOSITION OF BIOLOGICAL MATERIALS WITH AUTOMATED WET DIGESTION AND SUBSEQUENT Se DETERMINATION WITH FI-HG-AAS

Decomposition

Decomposition of samples is performed in open systems by automated wet ashing. The available sample amount is usually sufficient. Thus, 5 g organ tissue, wet weight and maximum amount of fat 500 mg (1 g organic tissue, dry weight) is suitable for wet ashing with 15 ml oxidizing acid mixture, $HNO_3/HClO_4$: 7/3 vol. per vol. (Frank 1976, 1983). For Se determination, in the routine procedure usually 1 g tissue wet weight is taken for wet digestion. For digestion of small amounts a semi-micro accessory was developed (Frank 1988).

Although work with perchloric acid is regarded as dangerous, in the standardized procedure perchloric acid has been used for more than 20 years without any disturbances. Rules and precautions have been introduced to make work safer. Some of the most important rules are described below.

A mixture of oxidizing acids is added to the samples; separate addition of acids must be avoided. Digestion is performed in tubes of borosilicate or quartz glass in an electrically heated block of aluminium connected to a microprocessor, which controls the programming of time and temperature (Tecator Digestion System, model 40, Höganäs, Sweden).

For most samples the standard program is used (Frank 1988) and the digestion is performed during the night. The temperature is fixed at 180°C in the morning. Tubes showing dark colour with visual control are removed from the block for repeated digestion. Tubes with clear solution are digested at 225°C for not longer than 30 min to prevent loss of Se.

Materials for which there is no previous knowledge about behaviour during digestion are treated by stepwise decomposition under visual control.

The hoods are made from polypropylene, the exhaust tubes and the fan are of PVC. From the hood up to the fan the whole system can easily be washed with water at any time. Thus, accumulation of dust or fumes of $HClO_4$ in the PVC tubes is prevented. In addition, directly on the hoods, scrubbers wash out the acid fumes from the air passing through the hoods. Four aluminium blocks in two hoods have a decomposition capacity of 4 x 40 samples per night.

It should be pointed out that the safety depends not only on rules, time- and temperature-controlled automated decomposition, special hood, exhaust system and sprinkler-washing system, but also on conscientious co-workers with a sense of responsibility.

At our department wet ashing of thousands of biological samples has been carried out with perchloric acid for more than 20 years, without any accident or explosion.

However, work with perchloric acid should only be undertaken if safety precautions are followed and care, caution, chemical knowledge and common sense are used.

Determination of Se

For determination of Se a method with the FI-HG-AAS technique was developed. FI-HG-AAS was used for the first time by Åström (1982) for determination of the hydride-forming element bismuth. Applications of this technique for Se determination are reviewed above.

Optimization

In both commercial and home-made equipment the systems are optimized to achieve maximum signal intensity and stability. Owing to the interactive character of the different instrumental and chemical parameters, the optimization becomes intricate.

Thus, the systems are optimized in respect to reagent concentration, carrier flow rate as well as gas flow rate. The length of reaction coil, different designs of gas-liquid separator and dimensions of quartz tube atomizer (cuvette) were also studied.

For the chemical reaction in the present dynamic system, optimum concentrations and relationship between HCl and $NaBH_4$, as well as flow rate of both, have to be found. Different concentrations of these reagents and conditions are described in the studies for Se determination with FI-HG-AAS (see above). In addition, carrier flow rate and gas flow rate are important parameters for optimization of the system.

Increased flow rate of the carrier results in increased sensitivity (Chan 1985;

Pettersson 1990; own experience). The simultaneously increased back pressure of the system can be controlled by stable tubing connections (own experience).

Control of gas flow rate and its fine adjustment with needle valves or mass-flow controller is important for optimization (Chan 1985; Galgan & Frank 1988; Negretti de Brätter et al. 1990).

The reaction of hydride generation is fast, but needs a definite time for reaction, and, for this reason, length and inner diameter of the reaction coil also play a role in optimization (Chan 1985; own experiences).

Different designs of gas-liquid separators are described in the literature. A stable gas flow into the quartz cuvette, minimized transport of liquid droplets and low dead volume are important parameters (Sturman 1985; Welz & Schubert-Jacobs 1991).

The dimensions of the quartz cuvette (atomizer) should be adapted to the AAS instrument with regard to the mechanical and optical conditions (Sturman 1985; Welz & Schubert-Jacobs 1991).

In order to achieve sufficient Se atomization in the quartz cuvette, some oxygen in the carrier gas is necessary, as well as an atomization temperature above 700°C (Agterdenbos et al. 1986). Generally, temperatures of 800-900°C are used.

Chemical interference

Chemical interference is well documented in the determination of Se with HG-AAS (Meyer et al. 1979; Welz 1985). Interference may be caused by other hydride-forming elements (As, Sb, Sn, etc) and certain metals (Co, Cu, Ni, etc). These effects are less pronounced in FI-HG-AAS than in HG-AAS because of the relatively low concentration of $NaBH_4$ and rapid transfer of SeH_2 from the sample solution to the gas phase, and, thus, from contact with interfering elements (Chan 1985; Wang & Fang 1986; Galgan & Frank 1988; Pettersson 1990; Welz & Schubert-Jacobs 1991).

Water is used as a carrier solution in the FI-HG-AAS system described by Welz & Schubert-Jacobs (1991). The advantages are a lower consumption of HCl and a less aggressive acidic environment. Furthermore, precipitation of interfering elements results eventually in depression of signal intensity and the need of a wash stage. Thus, in routine work an acidic carrier is preferable as it cleans the flow system continuously from precipitated elements (Chan 1985; Galgan & Frank 1988).

In biological materials disturbances from interfering elements are usually not serious, with the exception of those from copper. The liver from some Cu accumulating animals, inter alia sheep with chronic copper poisoning and mute swan, can contain Cu up to 1000 mg/kg wet weight or more. In our system when the sample solution contains > 1 μg Cu/ml, the solution has to be diluted and/or Fe(III) is added to reduce signal depression (Wang & Fang 1986; own experiences).

Equipment

The system used in our laboratory (see Fig. 1) is a combination of commercially available components (Galgan & Frank 1988), with the exception of the electrically heated oven and its power supply. During measurements the analog signal from the detector (AAS) is displayed by a recorder. The signal is converted simultaneously to digital form and the Se concentration in the sample solution is calculated by the FIA-star (Tecator AB, Höganäs,

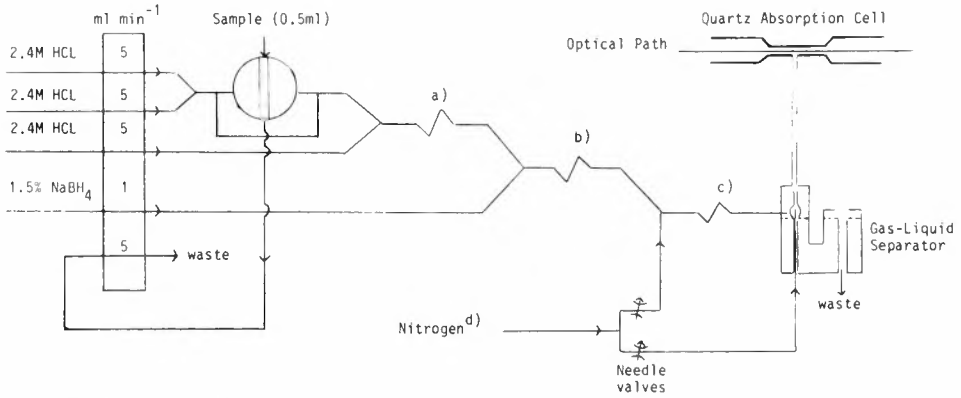

Fig. 1. Flow injection manifold for selenium determination by hydride generation and atomic absorption spectrometry (FI-HG-AAS)

Sweden). The results can be printed out or can be transferred to a computer for calculation of the results, as well as for collection and storage of data. However, use of a signal amplifier is necessary to raise the level from the FIA-star into the I/U-port of the computer.

By using any available AA-spectrometer, FIA-star with auto sampler, chemifold, gas-liquid separator (Varian), needle valves for regulation of gas flow, and a computer, the FI-HG-AAS system is totally automated. The maximum capacity is 120-180 measurements per hour (Galgan & Frank 1988). Moreover, the set-up does not require the continuous attention of an operator.

The system was described in 1988 and works successfully by using a Varian as well as a Perkin-Elmer atomic absorption spectrometer (Galgan & Frank 1988).

Advantages of the FI-HG-AAS system

The great advantages of the FI-HG-AAS system are the use of low sample volumes/amounts, the possibility to make repeated determinations, high sampling frequency, low reagent consumption, and the ease of automation. The system is characterized by high sensitivity and reproducibility in Se determination and relatively low sensitivity to interferences.

The sample amount, used in the digestion procedure, and the end volume after HCl reduction influence the limit of determination. When the weight of sample amount is 1.5-5 g, the limit of determination is 1-2 μg/kg (ng/g). However, decreasing sample weights (0.1 g) result in an increase in the limit of determination to 10 μg/kg (ng/g), if the end volume is not diminished at the same time. Thus, practical work is decisive for the limit of determination. The sensitivity of the method allows determination in the ppb range. The limit of determination is shown in Table 5. Selenium concentrations at the ppb level

(a few μg/kg) in milk, serum, plasma are not unusual in the Scandinavian countries. The accuracy of the method was checked by analysing standard reference materials. The results are set out in Table 6.

However, high sensitivity may cause problems when analysing samples with high Se concentrations because of difficulties in preparation of representative and homogeneous samples, as well as in handling great dilutions (see "The choice of the best method").

Table 5. Selenium: limit of determination in biological materials

Sample amount (g)	Oxidizing acid (ml)	Limit of determination (ng/g)	Materials low in Se (Scandinavian countries)
Wet weight:			
5.0	10	2	Milk (human, animal)
1.5	5	1	Plasma, serum (cow, sheep)
0.1	5	10	Muscle biopsy (horse, cow)
Dry weight:			
1.0	15	5	Flour
0.3	5	3	

Detection limit of the method: 0.070 μg/l (0.5 ml injection volume)
Characteristic concentration, sensitivity: 0.150 μg/l (A = 1%)
Capacity: 160-180 measurements per hour, 35-40 real samples

Table 6. Selenium concentration in standard reference materials

Standard reference material	Certif.[a]		Found [a]		n
Bovine liver (NBS 1577)	1.1	± 0.1	1.13	± 0.04 μg/g	5
Bovine liver (NBS 1577a)	0.71	± 0.07	0.70	± 0.04 "	5
Rice flour (NBS 11568)	0.4	± 0.1	0.38	± 0.06 "	5
Milkpowder (IAEA A-11)	0.0339	± 0.0072	0.0307	± 0.005 "	5
Animal muscle (IAEA H-4)	0.280	± 12%	0.300	± 0.008 "	5
Serum (Seronorm 105)	0.090	± 0.006[b]	0.090	± 0.004 μg/ml	8
Urine (Seronorm 108)	0.049		0.048	± 0.004 "	5

[a] 95% confidence limit of the overall means. [b] $\bar{x} \pm SD$

Setting up a system as described above requires knowledge and interest. It certainly takes more time to start work with a home-made system than with a commercial one. However,

by using existing available instruments the capital costs are lower and free choice of instruments results in increased flexibility.

THE CHOICE OF THE "BEST" METHOD

In all truthfulness, there is no best method of choice which can solve all the problems in the analysis of biological materials with different matrices and a broad range of concentrations. The choice of method is influenced by several parameters, depending on prerequisites such as economics, equipment, the problems to be solved, etc.

First of all, the analytical and practical questions should be elucidated and the requirements carefully analysed. The following parameters have to be taken into consideration:

- Type of material to be analysed. In the present discussion the material was given, i.e. biological material of animal and plant origin including organ tissues, but also natural and processed feed, mineral feed, as well as water, soil and probably environmental samples of different kinds.

- Quantity of available material. The amount of organ tissue from domestic and wild animals is often sufficient; however, from small animals this is limited and requires special procedures.

- Analyte concentration of the material. Variations of up to several orders of magnitude can exist. It is necessary to use methods with a limit of detection, well suited to the analyte concentration. Serious problems may arise when sensitive methods are used for materials with high analyte concentrations. In spite of dilution, the accuracy of the determination will not be as desired. Analysis of natural waters or plants growing on Se-depleted soils requires very sensitive methods and/or great volumes/amounts of sample material. Mineral feed may contain analyte in concentrations of a 3-5 times higher order of magnitude than organ tissues.

- The choice of sample pretreatment is of great importance. Different alternatives for decomposition have been discussed. Herewith, the completeness of decomposition, contamination problems, amount of sample and safety are all decisive for choice of method.

- Length of series to be analysed. In routine analysis long series of similar types of samples motivate the use of automated methods. In contrast, different kinds of samples with great variations in analyte concentrations demand more attention and individual treatment of samples.

- The personnel, economic resources and available equipment of the laboratory have to be taken into consideration.

- The choice of analytical instrument can be influenced by the need for flexibility, particularly in research in contrast to routine work.

- To perform simultaneous multielement analyses, use of ICP/DCP instruments appears to be convenient.

THERE IS NO BEST METHOD OF CHOICE, THE CHOICE OF METHOD IS YOURS!

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7 Analytical problems concerning glutathione peroxidase

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Several modifications of the coupled test method for the determination of glutathione peroxidase activity have been published. The results obtained by different methods are largely dependent on the assay conditions. Pretreatment of hemolysates with cyanide has been shown to reduce glutathione peroxidase activity in human and animal blood samples. The addition of glutathione restores enzyme activity during storage or cyanide treatment. The establishment of a recommended method for the determination of glutathione peroxidase activity would make the comparison of results from different studies comparable.

Key words: Assay methods, glutathione peroxidase, stability, substrates.

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Glutathione peroxidase activity (GSH-Px, EC 1.11.1.9) was first established in erythrocytes by Mills and co-workers in 1957. The properties of the enzyme were studied using purified GSH-Px isolated from bovine erythrocytes, and the enzyme was shown to have peculiar kinetics, so-called ping-pong kinetics. Saturation with the primary substrate glutathione (GSH) could not be achieved, and the apparent maximum velocities for the secondary substrate (H_2O_2, organic hydroperoxides) concentrations were a linear function of the GSH concentration (Flohé et al. 1972, Günzler et al. 1972). Thus, for assaying GSH-Px the most common method for determination of catalytic activity, by saturation of the enzyme with substrate, cannot be used. Several methods are described in which the assay conditions are selected to give a reasonable reaction rate with relatively low non-specific activity.

ASSAY METHODS

Glutathione peroxidase catalyses the reaction:

$$ROOH + 2\ GSH \ ----> \ GSSG + ROH + H_2O,$$

where GSH is oxidized in the presence of the secondary substrate, hydrogen peroxide, or organic hydroperoxides, e.g. cumene or tert-butylhydroperoxide. In a fixed time assay the

consumption of GSH is recorded over a short period. The method is mainly used for kinetic studies with purified GSH-Px, as crude biological samples often contain varying amounts of interfering components (Wendel 1980).

The coupled test system was first introduced by Paglia & Valentine (1967). GSH concentration was kept constant in the reaction mixture by using external NADPH and glutathione reductase (EC 1.6.4.2), which instantly reduces the oxidized GSH formed in the primary reaction:

$$2\ GSH + H_2O_2 \text{ --- GSH-Px ---> } H_2O + GSSG$$
$$GSSG + 2\ NADPH \text{ --- GSSG-reductase ---> } 2\ GSH + 2\ NADP^+$$

The enzyme activity is measured by constant monitoring of NADPH oxidation. The GSH-Px follows pseudo-zero order kinetics when the GSH concentration is kept constant in the assay. A generally accepted recommendation for the determination of GSH-Px activity has not been established. The results obtained by different methods are extensively dependent on GSH concentration in the assay and on the definition of the unit for the catalytic activity (Wendel 1980).

PROBLEMS IN ASSAYING BIOLOGICAL MATERIALS

The coupled test procedure proved to be suitable for determination of GSH-Px activity in biological samples without prior need to purify the enzyme. However, Paglia & Valentine (1967) observed that hydrogen peroxide could convert oxyhemoglobin to methemoglobin, and further oxidize NADPH nonenzymatically, or enzymatically by methemoglobin reductase. This non-specific oxidation of NADPH was prevented by converting the hemoglobin to cyanmethemoglobin in the hemolysate prior to determination of GSH-Px activity. Treatment of the purified GSH-Px with Drabkin's reagent, containing potassium cyanide and ferric cyanide, was not shown to inactivate the enzyme.

The coupled test was later improved by Günzler et al. (1974). Changing the secondary substrate hydrogen peroxide to tert-butylhydroperoxide, and the reaction temperature from 25 °C to 37 °C, decreased the non-specific catalytic reaction in the assay system and improved the stability of the substrate. Sodium azide, which was earlier used to inhibit catalase activity in the biological samples, could be omitted, when hydrogen peroxide was replaced with an organic hydroperoxide. Günzler and co-workers also confirmed that low concentrations of potassium or ferric cyanide did not inhibit the activity of purified GSH-Px.

Further studies on purified GSH-Px revealed an inhibitory effect of cyanide (Prohaska et al. 1977), particularly when the enzyme was in an oxidized form. Considerable loss of GSH-Px activity was also demonstrated in stored pig, cow and sheep blood (Blanchflower et al. 1986) when treated with cyanide prior to determination of GSH-Px activity. The authors managed to restore the enzyme activity by adding GSH to the hemolysate before treating it with cyanide solution. Flohé & Günzler (1984) also recommended preincubation of samples in assay mixture containing GSH to reactivate GSH-Px.

Except for GSH-Px, glutathione S-transferases also exert catalytic activity against

organic hydroperoxides, while hydrogen peroxide at low concentrations is a specific substrate to GSH-Px (Lawrence & Burk 1976). The activity of both enzymes, or only of GSH-Px, is recorded, depending on the substrate used in the assay. The relative proportions of GSH-Px and glutathione S-transferases vary between tissues within and between species, whereas the erythrocytes of various animal species contain solely GSH-Px (Lawrence & Burk 1978). Assay methods, using organic hydroperoxides as the substrate, indicate GSH-Px activity in tissues which have no glutathione S-transferase activity. However, if a more hydrophobic organic hydroperoxide, tert-butylhydroperoxide is used at low concentrations as the substrate, GSH-Px activity is preferentially assayed over glutathione S-transferases, which react with various organic hydroperoxides at higher concentrations (Burk et al. 1978).

STABILITY OF THE SAMPLES

Conflicting data have been reported concerning the effect of anticoagulants and storage time on GSH-Px activity. EDTA (ethylenediaminetetraacetic acid) has been preferred over heparin as an anticoagulant, as the latter was observed to have a strong inhibitory effect on GSH-Px activity (Günzler et al. 1974). In subsequent studies commonly used anticoagulants were shown to be suitable for GSH-Px determination (Sheppard & Millar 1981; Hussein & Jones 1981). The chemical quality of the heparin preparates might have contributed to the controversial results. The observed effect of storage on GSH-Px activity in blood samples seems to depend on the assay method used (Sheppard & Millar 1981; Hussein & Jones 1981; Blanchflower et al. 1986; Davidson et al. 1990). Omitting the cyanide treatment restores the GSH-Px activity in stored hemolysates (Blanchflower et al. 1986). The addition of GSH to porcine, bovine and ovine plasma samples prior to freezing was shown to prevent loss of GSH-Px activity during storage (Davidson et al. 1990).

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8 Diseases and diffuse disorders related to selenium deficiencies in ruminants

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Several diseases and negative production effects can be related to selenium deficiencies, but often other factors are aetiologically important as well. Since vitamin E is the most important of these factors, many researchers prefer to talk about "selenium-vitamin E responsive diseases". Among them, nutritional muscular dystrophy is the most well documented. Other health problems possibly related to selenium/vitamin E deficiencies are unthriftiness, reduced growth rate, retained placenta, impaired fertility and mastitis.

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When diseases and negative production effects related to selenium deficiencies are discussed, it is necessary to emphasize that several factors other than selenium can be involved in their aetiology. This is true for vitamin E in particular. Both selenium and vitamin E protect against free radical-induced, cell-destructive peroxidative reactions - vitamin E by sequestering free radicals and selenium (as a cofactor of the enzyme glutathione peroxidase, GSH-Px) by reducing hydrogen peroxide and lipid peroxides to water or harmless alcohols. The similar effects of selenium and vitamin E imply that an optimal level of one of them can alleviate the destructive effect of a deficiency of the other. Therefore, it is often impossible to state whether a beneficial effect in an experiment is due to the mineral, the vitamin or a combination of both. Many researchers therefore prefer to talk about "selenium - vitamin E responsive diseases" (Blood et al. 1988). So, although the primary aim of this article is to focus upon selenium, the role played by vitamin E cannot be excluded from the discussion.

The disorders which will be discussed are presented in Table 1.

Table 1. Health problems in ruminants related to selenium deficiency

Nutritional muscular dystrophy
Unthriftiness
Reduced growth rate
Retained placenta
Impaired fertility
Mastitis
Diarrhoea and pneumonia

NUTRITIONAL MUSCULAR DYSTROPHY (NMD)

Aetiology and pathogenesis

NMD is certainly the most well-documented disease among those that have been related to a deficient selenium status. Only one year after the discovery of the biological essentiality of selenium, a deficiency of the element was reported to be one of the main factors behind NMD in lambs (Hogue 1958; Muth et al. 1958). It became evident that NMD was common in areas where the dietary dry matter (DM) content of selenium was extremely low (<0.05 mg/kg). Muth (1963) reported that the disease could be prevented in lambs if the dietary intake was 0.10 mg/kg DM, a level which later was found to be somewhat too low (Whanger et al. 1977a). The preventive effect of supplemented selenium on the incidence of NMD has been verified by other researchers both in sheep (e.g. Drake et al. 1967; Hamdy et al. 1963; Evan et al. 1968) and in cattle (e.g. Hartley & Grant 1961). It thus seems indisputable that selenium defiency predisposes for NMD, particularly when the vitamin E supply is also inadequate, but other factors such as high levels of polyunsaturated fatty acids (PUFA) in the diet, unaccustomed exercice and rapid growth in young animals are often necessary as triggers in provoking clinical symptoms.

NMD in sheep (= white muscle disease, stiff lamb disease) occurs primarily in two age categories (Andrews et al. 1968). One critical period is during the first days of life. Lambs with a deficient selenium status are often born weak and may die within a few hours after physical exertion from running or sucking. Some lambs with NMD are even born dead. The second critical period is from one week up to three months of age, with a peak incidence at an age of between three and six weeks. Sporadic cases of NMD may also occur in older animals.

NMD in cattle is in many respects similar to the disease in sheep. It is thus mainly a disease of growing animals, although it is rare in newborn individuals. At high incidence of the disease has earlier been reported in one to two-month-old calves, which were fed solely on milk replacers containing high amounts of unsaturated fatty acids (Rosenberger 1978), but the incidence of this type of NMD has now been significantly reduced because of a more adequate composition of the milk replacers. One risk of today is fast-growing sucking calves of beef cows which graze on selenium-deficient pastures.

Another risk group is replacement heifers, which, after being fed extensively on a selenium and vitamin E-deficient winter diet are suddenly turned out to pasture. This last type of NMD has been analysed in more detail in some elegant studies by a research group from Belfast in Northern Ireland (Rice & McMurray 1982; McMurray et al. 1983; Kennedy et al. 1987) where it was found that when selenium and vitamin E-deficient calves were turned out on grass the concentrations of linolenic acid (C18:3) in plasma increased both absolutely and relatively (Fig. 1). At the same time the concentration of linoleic acid (C18:2) decreased, whereas the serum vitamin E concentration (Fig. 2) and the activity of the enzyme creatine kinase (CK) increased. The blood selenium concentration did not change significantly. The changed relation between C18:3 and C18:2 increases the risk of NMD since the peroxidizability rates for C18:3 are twice those for C18:2 and about 25 times higher than those for C18:1 (McMurray et al. 1983). Since an increased CK activity indicates destruction of muscular tissue, it is evident that the increased dietary intake of vitamin E from the grass did not prevent the NMD-provoking action of C18:3 during the

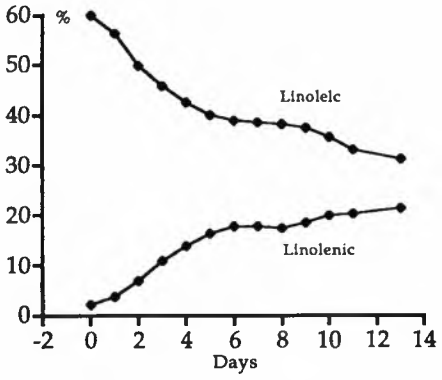

Fig. 1. Mean changes in plasma linoleic (C18:2) and linolenic (C18:3) acids (% of total fatty acids) in calves turned out to spring pasture on day 0 (McMurray et al. 1983)

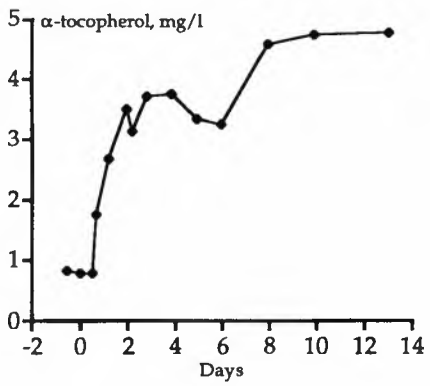

Fig. 2. Mean changes in plasma α-tocopherol in calves turned out to pasture on day 0 (McMurray et al. 1983)

very first days on pasture. However, in later experiments the authors found that NMD could be prevented when a surplus of vitamin E was given to the animals a few days before they were turned out. They concluded that the rates of deposition of PUFA and α-tocopherol on key tissue sites are probably different. In other words, in a selenium and vitamin E-deficient animal PUFA are peroxidized within a short time after being absorbed, whereas it will take somewhat longer for the fat-soluble vitamin to reach the site for its action in the cell membrane. In the meantime the animal will be highly predisposed to NMD.

The presented hypothesis for the pathogenesis of NMD was later verified experimentally by the same research group. Selenium and vitamin E-deficient indoor-fed calves were given vitamin E-stripped linseed oil with a fatty acid composition closely resembling that of grass (high content of C18:3 and low content of C18:2) and chemically protected from being hydrogenated in the rumen. The CK activity in plasma increased within a few days simultaneously with the concentration of C18:3, and histopathological investigations revealed degenerative processes in the muscles of the same type as those found in spontaneous NMD.

It can thus be concluded that NMD can be provoked in selenium and vitamin E-deficient animals without increased muscular activity. However, the importance of sudden muscular acitivity when the animals are turned out to grass as a trigger factor for NMD has been emphasized by Arthur (1982) and Pehrson et al. (1986). Pehrson et al. found significantly lower mean vitamin E values in serum in clinically diseased calves (0.79 mg/l) than in healthy calves grazing the same pasture (2.14 mg/l), which indicates that the calves became ill so soon after having been turned out that their consumption of grass in fact must have been very low. If so, their dietary intake of PUFA must have been much lower than that in the experiments by the Belfast group, since Swedish fresh grass also has a much

higher C18:3 and vitamin E content than hay and other feedstuffs used during the stall period (Hakkarainen & Pehrson 1987).

Clinical findings

In peracute cases it is not unusual that the animals die suddenly without showing any preceding clinical signs. In these cases the myopathy is primarily located in the myocardium. However, in the most common form of the disease affected animals have a stiff gait and an arched back, and are unwilling to move. Muscular tremor may occur and the affected muscle masses tend to be swollen and firmer than normal. If the diaphragm and intercostal muscles are involved, dyspnea and respiration of abdominal type will occur. In severe cases the animal may become prostrate and die. The temperature is usually normal. Myoglobinuria is more common in older than in newborn animals and will, according to results presented by Holmgren (1988), probably disappear within one to two days if no continuous muscular destruction occurs.

Clinical pathology

Plasma or serum CK activity is the most commonly used laboratory aid in the diagnosis of NMD. It remains a good diagnostic tool for at least three days after onset of muscular damage (Blood et al. 1988; Holmgren 1988). The CK activity is proportional to the extent of the damage. Pehrson et al. (1986) reported that healthy grazing calves had less than 5 μkat/l serum, compared with 200-400 μkat/l in subclinically diseased calves and with about 1000 μkat/l in clinically diseased calves, but even higher values can be found in severe, acute cases (Rice & McMurray 1982).

According to common handbooks (Rosenberger 1978; Blood et al. 1988) whole blood selenium values in animals with clinical NMD typically are 5-20 μg/l (whole blood values are 1.5-2.5 times higher than those of serum or plasma). Such values are without doubt extremely low. Somewhat higher values (up to 20 μg/l serum) have been reported by Whanger et al. (1977a) and by Pehrson et al. (1986), who found 25-30 μg/l whole blood, but even these concentrations are certainly deficient.

GSH-Px activity is often used instead of selenium analyses in whole blood, serum or erythrocytes for evaluating the selenium status of an animal. GSH-Px values must, however, be evaluated according to the correlation curves found between selenium and GSH-Px for each laboratory.

A liver selenium concentration of less than 0.20 mg/kg wet weight (w.w.) is considered to indicate a deficiency (Blood et al. 1988). Very low concentrations (<0.10 mg/kg w.w.) are often found both in cattle and sheep with NMD (Andrews et al. 1968; Frøslie et al. 1979).

Necropsy findings

The lesions in skeletal muscles are always bilaterally symmetrical. The large muscles of thigh and back, the intercostal muscles and the diaphragm are usually affected. In these muscles white or greyish areas can be seen ("fish flesh"). If myocardium is involved, white areas of degeneration can be seen particularly in the left ventricle and papillary muscles. Typical hyaline degenerations and coagulative necroses can be found at histological examination.

Treatment

Animals with NMD should be kept isolated to avoid unnecessary muscular exertion. About 6 mg selenium/100 kg body weight (preferably as sodium selenite) should be given intramuscularly or subcutaneously together with vitamin E (at least 300 mg/100 kg body weight). It is also recommended that additional vitamin E be given orally in the course of the next three to four days.

Prevention

As mentioned above, McMurray et al. (1983) found that supplementation with vitamin E can prevent NMD in selenium-deficient calves, and Whanger et al. (1977b) evaluated vitamin E to be more effective than selenium for prevention of NMD in sheep. Other researchers have found that vitamin E, when given alone, was ineffective for the prevention of NMD in selenium-deficient newborn lambs (Drake et al. 1960; Muth et al. 1961). In the last-mentioned studies, however, the vitamin supplementation was given to pregnant ewes, and it is now well known that vitamin E, in contrast to selenium, only to a very limited degree passes across the placental barrier. The interpretation of the results is further complicated by the fact that vitamin E-treated pregnant ewes have a higher concentration of vitamin E in colostrum (Pehrson et al. 1990).

The preventive measures against NMD should be adapted to the selenium and vitamin E status of the herd in question. If the dietary vitamin E supply is satisfactory, a selenium concentration in the total feed DM of 0.2 mg/kg is considered to be sufficient (Blood et al. 1988). If the natural feed ingredients are lower in selenium, dietary supplementation is needed. The most common supplement is sodium selenite. It is also possible to use organic selenium (preferably selenium yeast), but new scientific data indicate that the metabolic pathways for organic and inorganic selenium compounds are so different that the dose should be different (Hakkarainen 1993; Pehrson 1993).

Parenteral administration of selenium seems to have no advantage over dietary supplementation for prevention of NMD.

UNTHRIFTINESS (= ILL-THRIFT)

Unthriftiness is described particularly in sheep from New Zealand, where it has been considered the most widespread and economically important of all selenium-responsive diseases (Andrews et al. 1968). It has also been reported from Australia and USA, but for unknown reasons the preventive effect of selenium administration in these countries has been smaller than that in New Zealand (Blood et al. 1988).

Affected lambs appear to be quite normal during their first two to three months of life, but after that age they display a poor appetite and reduced growth rate. Some of them even lose weight, become dejected and die. Occasionally, diarrhoea might occur. In ewes infertility is a common feature. It is interesting to note, however, that whereas selenium supplementation to ewes on unthriftiness - provoking pastures has resulted in increased fertility (Hartley & Grant 1961), no adverse effects were found on conception rates, embryonic mortality or numbers of lambs born in ewes on an experimentally selenium-depleted diet (Mitchell et al. 1975). This indicates that other factors besides selenium

deficiency may be involved in the ill-thrift complex. According to Hartley & Grant (1961) vitamin E is not likely to be such a factor, since vitamin E-supplemented animals did not give results distinguishable from those of undosed controls.

Unthriftiness in young cattle can occur at different ages. The clinical symptoms vary from different degrees of suboptimal growth rate to progressive loss of condition and profuse diarrhoea.

Selenium-responsive unthriftiness has been found only on pastures containing very low selenium levels (<0.02 mg/kg DM; Blood et al. 1988). On the same type of pastures NMD is common, but the presence of severe unthriftiness in some areas with no NMD problems (Hartley & Grant 1961) again indicates that other factors may be involved.

The selenium concentration in whole blood is very low in manifest cases of illthrift - mostly lower than 10 μg/l (Blood et al. 1988). This further indicates that unthriftiness in its typical form is a disease complex that is related to very pronounced selenium-deficient conditions.

The post-mortem findings in unthriftiness are non-specific and just reveal emaciation. There are no characteristic histopathological lesions.

REDUCED GROWTH RATE

As mentioned above, reduced growth rate is a predominant feature in unthriftiness. There are several other reports on reduced growth rate in young animals given selenium deficient diets, where an association with the clinically more serious condition defined as unthriftiness has not been made. It might, however, be relevant to look upon these cases as less serious forms of unthriftiness. Oldfield et al. (1960) reported that lambs from selenium-supplemented ewes grew almost twice as much during the first six weeks of life as lambs from selenium-deficient ewes. These results were confirmed by Rotruck et al. (1969), who found a significantly higher weight gain in lambs from ewes given diets containing 0.2 and 1.0 mg selenium/kg than in lambs of ewes given only 0.02-0.03 mg selenium/kg. Positive effects of selenium supplementation on growth rates have also been reported in cattle when the basic diet contained less than 0.05 mg selenium/kg (Gleed et al. 1983). The deficient state of the non-supplemented animals in this study was confirmed by very low whole blood selenium concentrations (<20 μg/l). After two injections of selenium the blood values were increased to about 60 μg/l. The treated animals grew 11-16 kg more than the controls during the grazing season. In contrast, Weiss et al. (1983) and Swecker et al. (1989) did not find increased growth rates in sucklings and weaned calves, respectively. Even though the basic diets were considered by the authors to be deficient in selenium, it is interesting to note that the whole blood/serum selenium concentrations were considerably higher than those in the trials reported by Gleed et al. (1983). Thus, Weiss et al. (1983) found serum selenium values of 19-24 μg/l and Swecker et al. (1989) whole blood values of 66-79 μg/l. However, it must also be mentioned that Shirley et al. (1966) did not find any positive effect on growth rate in calves after intramuscular administration of selenium to their mothers even though the pasture used only contained 0.02-0.06 mg selenium/kg DM. Arthur et al. (1988) proposed that some of the adverse effects of selenium deficiency on growth may be mediated by disturbances in thyroid hormone

metabolism, but found in their own experiment that severely selenium-deficient calves (mean dietary concentration 0.02 mg/kg; mean whole blood concentration 8 μg/l) had exactly the same growth rate as selenium-supplemented calves (mean dietary concentration 0.12 mg/kg; mean whole blood concentration 81 μg/l).

As a general conclusion it seems that the growth rate of young ruminants may be reduced at severe levels of selenium deficiency. At marginal or optimal dietary selenium levels it can be assumed that there is no significant reduction.

RETAINED PLACENTA

In a trial with a total of 171 cows Trinder et al. (1973) reported that an incidence of retained placenta of 39% could be reduced to 10% after injecting selenium one month before expected calving and to 2% if vitamin E was given together with selenium. The dietary selenium level in the herd was 0.03-0.05 mg/kg DM and the mean whole blood selenium concentration in dry cows before the injection 68 μg/l. Harrison et al. (1984) found an incidence of retained placenta of 16% in unsupplemented control cows with an average plasma selenium concentration of about 30 μg/l. After selenium and vitamin E supplementation the incidence was 0%, but there were no differences between the controls and cows treated with either vitamin E or selenium. Julien et al. (1976) found that the incidence of retained placenta was reduced from 38% to 0% when the dietary selenium concentration was increased from about 0.02 to 0.08 mg/kg DM. However, the study comprised only 42 cows. The selenium concentrations in plasma at the two dietary levels were 25 μg/l and 85 μg/l, respectively. In field experiments including a total of 2595 cows an injection of selenium and vitamin E three weeks prepartum reduced the incidence from 26% to 13% with large variations in effect between different states in the USA (Conrad 1985). No blood or dietary selenium values were presented in this trial. Bostedt & Schramel (1983) reduced the incidence of retained placenta from 20% to 5% in a trial comprising 234 cows by injection of selenium two weeks before parturition. In a three-year study in one herd the incidence of retained placenta was reduced from 25% among 487 cows to 13% among 614 cows when injections of selenium and/or vitamin E were given three weeks before calving (Eger et al. 1985). Selenium alone was at least as effective as a combination of selenium and vitamin E. The dietary selenium level in the prepartum diet ranged from 0.04 to 0.11 mg/kg.

Other researchers did not find any positive effect of addition of selenium on the incidence of retained placenta. However, in the studies by Ishak et al. (1983) and Hidiroglou et al. (1987) - who found incidences of 28% and 21%, respectively - the dietary selenium levels were adequate (0.21 and 0.1-0.2 mg/kg, respectively) as were the serum/plasma selenium concentrations ($\bar{x}$, 80 and 71 μg/l, respectively). Segerson et al. (1981) found corresponding incidences in selenium + vitamin E-supplemented and in non-supplemented animals ($\bar{x}$, = 17 and 19%, respectively). They considered 83% of the 440 cows studied to be selenium-deficient before the supplementation. However, their borderline values are subject to discussion, since they considered serum selenium concentrations lower than 80 μg/l to be deficient.

Retained placenta occurs normally in 5-10% of parturient dairy cows. It seems

relevant to conclude that a selenium deficiency may be involved in the aetiology if the incidence is considerably higher than that. It is evident, however, that other aetiological factors can cause increased frequencies. A deficient selenium status of a herd should therefore be established before supplementation with the element. At normal incidences there seems to be no reason to supplement the diet with selenium or to inject selenium salts as a prophylactic measure.

IMPAIRED FERTILITY

The possible effect of a selenium deficiency on fertility has been studied by many researchers. Hartley & Grant (1961) in New Zealand and Godwin et al. (1970) in Australia both found a significantly increased fertility when selenium deficient ewes (whole blood selenium <20-35 μg/l) were supplemented with selenium. When whole blood selenium concentration was higher before supplementation (80-120 μg/l) the reproduction in the ewes was not increased, but there was a higher preweaning survival in the lambs after supplementation (Kott et al. 1983). In other trials with sheep no effects of selenium supplementation were found (Davies 1966; Maxwell 1972), but no information is given in these publications about the basic dietary selenium levels.

There are many reports from trials with cattle in which selenium supplementation failed to affect reproductive performance. In several of these trials, however, the selenium content of the basal diet was sufficient (Ishak et al. 1983; Kappel et al. 1984; Hidiroglou et al. 1987). Larson et al. (1980) found a positive correlation between serum selenium and days open within selenium sufficient cows and Ropstad et al. (1987) reported a tendency towards a better reproductive performance in herds with a mean whole blood selenium concentration of 100 μg/l compared with herds with a mean concentration of 150 μg/l. Mohammed et al. (1991) did not find any difference in whole blood selenium concentrations between cows with and without cystic ovaries (141 and 136 μg/l, respectively), but a multivariate logistic regression analysis revealed that cows with whole blood concentrations higher than 169 μg/l had twice the risk of cystic ovaries compared with cows with selenium levels less than 108 μg/l.

A positive effect on conception rate to first service (from 51% to 69%) was reported by Tasker et al. (1987) when a long-lasting selenium injection was given to cows in two herds with extremely low mean whole blood selenium concentrations (9-10 μg/l). In another herd, however, no effect was achieved although even the cows in this herd had a very low mean whole blood selenium level (16 μg/l).

Based on the mentioned references a positive effect on fertility by supplementation of selenium can be expected only in extremely selenium-deficient animals. In such cases a positive effect might be due to an increased fertilization of ova as indicated by experiments in superovulated beef cows (Segerson et al. 1977).

MASTITIS

The risk of an increased incidence of mastitis (and other infectious diseases) at selenium deficiency might be related to the role of selenium in maintaining an optimal immune

function. Selenium deficient animals produce less immunoglobulins and fewer antibody-forming cells on antigenic challenge. Moreover, the efficiency of oxygen consumption and bactericidal activity in neutrophils is decreased (Burkholder & Swecker 1990).

The relations between selenium (and vitamin E) deficiency and mastitis have been studied mainly by two research groups - one in Ohio and one in Pennsylvania. Smith et al. (1984) reported a 12% reduction (not significant) of clinical mastitis during lactation after injection of selenium during the dry period. The reduction was 37% (significant) in vitamin E-supplemented cows and there was no further effect from a combination of selenium and vitamin E. The duration of clinical symptoms was reduced by 46% in the selenium group and by 44% in the vitamin E group. The material was small, just 20 cows in each group. Weiss et al. (1990) reported that both bulk tank somatic cell counts and clinical mastitis were negatively correlated to plasma selenium concentration. However, as can be seen in Fig. 3 the material was small. Erskine et al. (1987) reported that 16 herds with low 12-months' mean somatic cell counts (< 100 000 cells/ml) had a better selenium status than 16 herds with high cell counts (> 700 000 cells/ml). The mean whole blood concentrations of selenium were 133 and 74 μg/l, respectively. Furthermore, Braun et al. (1991) found that nine herds with "chronical mastitis problems" had significantly lower mean serum selenium concentrations (10 μg/l) than 33 "healthy" control herds (18 μg/l). Both of these herd categories were thus selenium deficient.

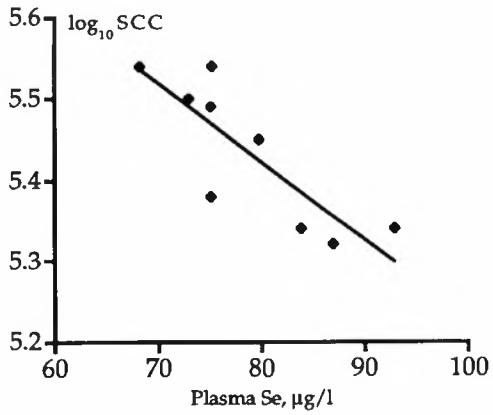

Fig. 3. Relationship between herd mean selenium concentration in plasma and bulk tank somatic cell counts (SCC) in nine herds (Weiss et al. 1990)

When Erskine et al. (1989) in the fourteenth lactation week challenged 10 selenium-deficient heifers (mean whole blood selenium concentration 33 μg/l) and 10 selenium-supplemented heifers ($\bar{x}$ = 132 μg/l) by intramammary *Escherichia coli* administration, infection was established in all animals, but there was less atrophy and agalactia, lower cell counts, lower peak bacterial concentration and a shorter duration of infection in the selenium-supplemented group. When the same experimental model was applied on heifers using *Staphylococcus aureus* (Erskine et al. 1990), the selenium status did not affect the infection rate or the duration and severity of the mastitis. However, the authors suggested that there was in fact some evidence of enhanced mammary resistance in the selenium-supplemented animals, because peak bacterial concentration was lower and was attained later than in the selenium-deficient group. These results can be related to those from three other independent studies, showing that there were no differences between the ability of the polymorphonuclear leucocytes from selenium-deficient and selenium-supplemented animals to phagocyte mastitis-provoking bacteria, but a significantly better ability to kill the phagocytized bacteria in the leucocytes from the selenium-supplemented animals (Gyang et al. 1984; Grasso et al. 1990; Hogan et al. 1990).

In conclusion, there are clear indications that selenium deficiency to some extent can

decrease the resistance against mastitis. However, most of the documentations are based on limited material and on experimental or *in vitro* studies and their practical relevance must therefore be further studied. Finally, it can be noted that Ropstad et al. (1987) in a field study found a tendency towards a higher frequency of mastitis in herds with a sufficient selenium status than in herds with a marginal selenium status.

DIARRHOEA AND PNEUMONIA

The negative influence of a selenium deficiency on the immune competence should increase the risk of many other infectious diseases besides mastitis. However, there are only a few publications in this domain. Moser et al. (1978) found a decreased incidence of pneumonia and scours in neonatal calves when they supplemented a presumed selenium-deficient whole milk diet with selenium. On the other hand, no positive health effects have been reported from selenium-supplementation of deficient calves (Weiss et al. 1983; Phillipo et al. 1987) or heifers (Sørensen et al. 1983). Droke & Loerch (1989) reported that although the serum antibody response to *Pasteurella haemolytica* vaccination was enhanced in selenium-deficient steers, their performance and health status were not affected.

CONCLUSIONS

There is no doubt that a selenium deficiency is of great importance for nutritional muscular dystrophy (NMD) and unthriftiness. The risk of these diseases increases with the degree of deficiency. Selenium deficiency can also give rise to reduced growth rate and increase the risk of retained placenta. *In vitro* and experimental studies indicate a lowered resistance against infectious diseases in selenium deficient animals. However, the clinical importance of these findings are far from convincing concerning mastitis, and for other infectious diseases and fertility most of the trials have not revealed any clinical importance at all.

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9 The pathology of diseases and diffuse disorders due to selenium deficiency in non-ruminants

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Vitamin E and selenium have important roles in the proper functioning of a wide variety of cells, tissues, and organs. These tissues and organs in turn affect many physiological and biochemical reactions. Of special interest is the role of vitamin E and selenium in resistance to infection and toxicosis, enhancing immunity, and inhibiting the effects of toxin and endotoxin production from the intestinal microflora. A number of diseases have been noted to respond to vitamin E-selenium therapy. The manipulation of cellular levels of selenium may be significant for the maintenance of general health and for the control of immunodeficiency disorders. As additional information is obtained, these nutrients will be recognized for their important role in all livestock production and for improving human health as well.

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Selenium (Se) and vitamin E play an important role in efficient, profitable animal production and in maintaining optimum health. Vitamin E and Se are considered together because they both function as antioxidants and each has additional specific functions related to maintaining health. Vitamin E acts predominantly in cellular membranes, and Se acts in the glutathione peroxidase system of intracellular components. Numerous factors connected with vitamin E-selenium influence their requirement and metabolism, as well as the clinical signs and lesions of deficiency.

Pigs may show a variety of deficiency diseases that are related to their nutritional status with respect to Se, vitamin E, antioxidants and polyunsaturated fats. These diseases include hepatic degeneration, mulberry heart disease, microangiopathy and skeletal muscle dystrophy. Altered epithelial cell morphology and reduced tolerance to parenterally administered iron have also been reported as consequences of Se/vitamin E-deficiency.

The effects of Se on the ability of Se-deficient or -supplemented animals to mount an inflammatory response and to influence the a humoral and some cell-mediated immune responses have been reviewed (Spallholz 1981). Accumulated evidence indicates that Se deficiency in animals is associated with an impairment of immune responsiveness and that Se supplementation enhances the depressed response resulting in an increase in immunologi competence.

Controversy still exists, though, regarding vitamin E-selenium because of the complexities of the problems. As new information is obtained from experimental work, contro-

versies will diminish and real progress will be achieved. The purpose of this report is to give an overview of the pathology of the diseases attributable to Se-E deficiency in domestic animals.

HEPATOSIS DIETETICA

The combined deficiency of Se and vitamin E in the pig frequently leads to hepatic degeneration, referred to as hepatosis dietetica (Obel 1953). The condition was first produced experimentally in vitamin E-deficient swine but was subsequently described in the field. Hepatosis dietetica shows two clinical patterns: an acute type with acute liver failure usually in rapidly growing young pigs and resulting in sudden death, and a subacute type with ascites and jaundice, which generally accompanies edema and/or cardiomyopathy (Hakkarainen et al. 1978). The acute lesions are characterized by multiple scattered swollen lobules, with necrosis, and edema of the wall of the gallbladder. Microscopic examination reveals massive necrosis often with hemorrhages. The necrotic processes may occur only in the central part or extend over the entire lobule without passing the borders of the acini (Fig. 1). The subacute and chronic lesions appear as scattered collapsed lobules that yield a rough and granular appearance to the surface of the liver with some lobules remaining unaffected (Fig. 2).

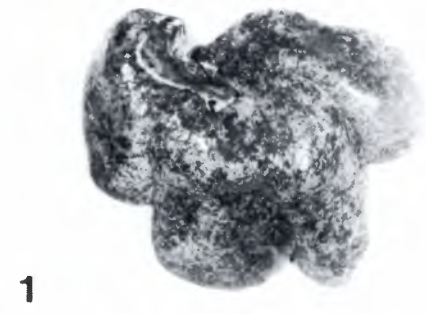

Fig. 1. Acute stage of hepatosis dietetica. The liver has mottled pattern due to widespread massive necrosis

Fig. 2. Chronic hepatosis dietetica. The liver displays numerous grooves and has a granular appearance as a result of post-necrotic scarring

MULBERRY HEART DISEASE AND MICROANGIOPATHY

Growing pigs fed Se- and vitamin E-deficient diets can develop a severe cardiomyopathy characterized by epicardial and myocardial hemorrhages. The widespread appearance of hemorrhages in this condition suggested the name mulberry heart. This condition frequently occurs in pigs with subacute hepatosis dietetica, but may occur without hepatic involvement. In addition to the signs already indicated, affected animals show scattered pale streaks on the ventricular myocardium and abundant serous fluids in the body cavities with pulmonary congestion and edema. Microscopic examination reveals both vascular and

myocytic lesions. Grant (1961) conducted a detailed study of micropathology of the disease, and because the primary lesions were in the small arterioles and capillaries and of dietary origin, he termed the disease dietetic microangiopathy. In addition to the heart, widespread distribution of extracardiac microangiopathy was found in several organs and tissues.

The vascular lesions include fibrinoid necrosis in intramyocardial small arteries and arterioles with fibrin microthrombi in the myocardial capillaries. This results in myocardial hemorrhages and edema. Multifocal hyaline necrosis of myofibers are observed throughout the heart but are most severe in the atria (Fig. 3). Pigs that survive for prolonged periods of time display myocardial fibrosis with macrophage infiltration (Bengtsson et al. 1978a). The ultrastructural basis for myocyte lesions appears to include mitochondrial swelling and mineralization, myofibrillar lysis, and contraction band necrosis (Van Vleet et al. 1977a). Although vascular lesions are present the fiber alterations develop independently of the vascular changes. Affected vessels show endothelial cell damage and necrosis with fibrin accumulation (Van Vleet et al. 1977b).

MUSCULAR DYSTROPHY

Pigs

Skeletal myopathies are frequently observed in Se- and vitamin E-deficient pigs with hepatosis dietetica and/or mulberry heart disease (Bengtsson et al. 1978b). Affected pigs display generalized muscular weakness and walk with an unsteady gait. In some cases, lesions may be apparent upon gross examination as areas of pallor, most commonly in the quadriceps femoris, gracilis, adductor, psoas and longissimus dorsi muscles. Microscopic examination reveals hyaline degeneration with loss of striations, vacuolization, and disruption of muscle fiber groups. Subsequent macrophagic invasion and phagocytosis of disrupted sarcoplasm is followed by muscle fiber regeneration (Fig. 4). The earliest electron microscopic alterations are myofibrillar lysis and disruption of mitochondria, sarcoplasmic reticulum, and plasma membranes (Van Vleet et al. 1976). Indicative of muscle degeneration is the increase in the activity of creatine phosphokinase observed in serum from Se- and vitamin E-deficient pigs affected with nutritional muscular dystrophy.

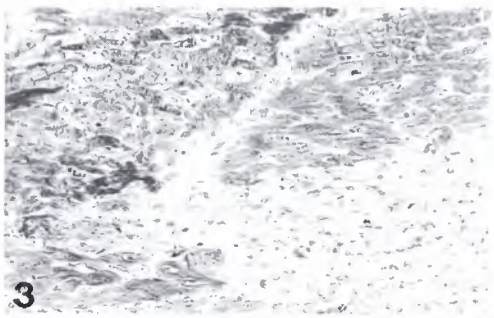

Fig. 3. Mulberry heart disease. Myocardium with both acute degenerative changes of myofibers (upper left) and reparative processes with proliferation of histiocytes and fibroblasts (lower right). Gomori's trichrome x 160

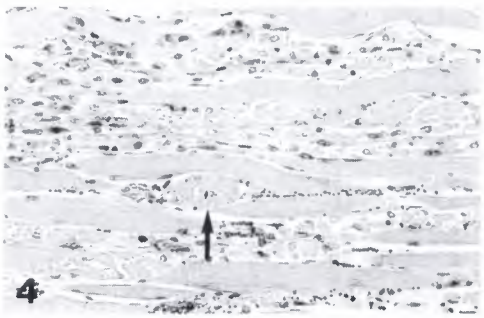

Fig. 4. Muscular dystrophy in a pig. Normal muscle fibers are found interspersed with fibers of hyalin fragmentation and lysis. Macrophagic invasion and phagocytosis are evident and microangiopathy is indicated by an arrow. HE x 340

Chickens

Muscular dystrophy occurs in the growing chick, often in combination with exudative diathesis (Kristiansen 1973) but only exceptionally in connection with encephalomalacia (Marthedal 1973). The disease is characterized by degeneration of the skeletal muscles, which is especially prominent in the pectoralis and gastrocnemius. It is seen as longitudinal white striations in the muscle fibers, usually visible through the skin. The earliest detectable lesions at the ultrastructural level are seen in the vascular bed, and neuromuscular junctions (Sweeny & Brown 1980). Dietary supplements of Se are effective in reducing but not fully preventing nutritional muscular dystrophy in the chick. Calvert & Scott (1963) demonstrated that although supplemental Se markedly reduced the amount of vitamin E required by the chick for preventing myopathy, it did not affect the level of cystine needed for similar protection.

Horses

Various muscular lesions in horses such as polymyositis, paralytic myoglobinuria and muscular dystrophy have been known and described since the beginning of this century. The view that a vitamin E and selenium responsive form of muscular dystrophy occurs in horses is supported by more recent papers (Schougaard et al. 1972; Ronéus & Jönsson 1984). The disease is observed in young foals between a few days and 10 weeks of age but also in adult horses (Owen et al. 1977). Affected animals develop general weakness, stiffness of gait, and dyspnea. After the first signs have been observed the disease may take a very rapid course, and the animals may die within a few hours or days. Myoglobinuria may be present. The gross and microscopical changes of the skeletal muscles resemble those of nutritional muscular dystrophy in other species. The heart is often involved and dystrophic calcifications are frequently seen. The disease pattern of muscular dystrophy foals thus resembles that associated with selenium deficiency in young ruminants.

GASTRIC ULCERS

Ulcers in the cutaneous mucous membrane of the stomach of young pigs are often observed in experiments designed for studying Se- and vitamin E deficiency (Sharp et al. 1972; Bengtsson et al. 1978a) and in field cases of hepatosis dietetica and mulberry heart disease. Many pigs die suddenly owing to massive i hemorrhage into the stomach. The most frequent early indication of subacute or chronic forms of the diseaseis in bloody feces. The postmortem picture shows that ulcer development appears to start with epithelial changes such as parakeratosis and focal surface erosions. The degenerated epithelial layer is eroded and the ulceration may extend further into the ventricular wall with subsequent development of bleeding ulcer. Microangiopathy and thrombosis of small vessels, which is often seen in gastrointestinal tissue, may also contribute to the pathogenesis of gastric ulcers.

EXUDATIVE DIATHESIS

The combined deficiency of Se and vitamin E produces exudative diathesis in young chicks within 2-4 weeks when they are reared with deficient diets from the time of hatching. Both

the incidence and rate of appearance of the disease are dependent upon the Se-vitamin E status of the flock. Exudative diathesis is characterized by varying degrees of subcutaneous, intermuscular and interstitial edema, particularly abdominal and ventral aspects of the neck and wings. The edema soon progresses to a hemorrhagic stage, producing a blue-green discoloration of the skin. Microscopic examination reveals capillary and venous thrombosis and fibrinoid degeneration of arterioles. The exudation apparently results from abnormally increased permeability of the damaged vessels.

ENCEPHALOMALACIA

Encephalomalacia is seen in young chickens, predominantly at the age of 3-8 weeks. Apparently healthy birds suddenly become atactic and the majority of affected animals die within a couple of days. Postmortem changes are hemorrhages, edema and necrosis of the cerebellum, cerebrum and sporadically in other parts of the brain. The earliest histopathological changes are seen in the cerebellum, characterized by separation of the molecular and granular layers. Hyaline thrombosis in the capillaries with hemorrhages and edema are observed in the cerebellum but also in other parts of the brain with subsequent development of ischemic necroses. The disease is produced by deficiency of vitamin E in chick diets containing no synthetic antioxidants. Selenium is found to be without protective effect against encephalomalacia (Yoshida & Hoshii 1977).

OTHER DISORDERS

Resistance to toxic chemicals

It is well established that combined Se- and vitamin E-deficiency can reduce the tolerance of baby pigs to parenteral iron supplements. Iron-induced myopathy and death among Se- and vitamin E-deficient pigs are reported (Lannek et al. 1962; Patterson & Allen 1972). In additional studies Se and vitamin E supplementation was protective against toxicity of silver, cobolt, tellurium, zinc, cadmium and vanadium in pigs (Van Vleet et al. 1981). Thus, it is evident that in the pig suffering from selenium-vitamin E deficiency, susceptibility to chemical or toxic substances is increased and supplementation with vitamin E-selenium is at least partially protective.

The role in immunity and infection

Norwegian researchers conducted a series of experiments to determine whether vitamin E-selenium-deficient pigs were more susceptible to swine dysentery. They found that selenium alone or with vitamin E reduced mortality, days of anorexia and diarrhea and improved weight gains (Teige et al. 1982). In an earlier report, it was suggested that vitamin E status may play a role in the manifestation of the Schwartzman reaction and the etiology of swine dysentery (Nordstoga 1973). This was confirmed by Teige et al. (1984) who showed that Se is more important than vitamin E in this respect. Although the mechanism is unclear for the protection by dietary Se against swine dysentery, it was suggested that Se may act by stimulating the non-specific immunity of the colonic mucosa.

The metritis-mastitis-galactia complex (MMA), or agalactia toxemica (Ringarp 1960), is another important problem in sows at parturition. Reports have stated that the inconsistent recovery of infectious agents from the mammary glands and uterus and the lack of a predominant infectious agent in these tissues suggest that the MMA syndrome is not an infectious disease per se (Martin et al. 1967; Trapp et al. 1970). Experiments were conducted to confirm the value of vitamin E-selenium in preventing MMA. The results showed that vitamin E- and selenium-deficient swine, with the nature of their tissue degeneration and impaired neutrophils, are susceptible to any environmental pathogens as secondary invaders.

It is proposed that the vitamin E deficiency affects mainly the platelet system with both a production and function defect, while the selenium deficiency affects the fibrinogen system, causing low-grade disseminated intravascular coagulation (Fountaine et al. 1975). Thus, the administration of an endotoxin in the mammary gland (as done experimentally) could enhance a pathological process (Nachreiner et al. 1972). Other bacterial toxins could also enhance the injury.

Supplemental vitamin E improved the humoral immune response of mice, chickens, turkeys, and guinea pigs exposed to various antigens and increased the resistance of chickens and turkeys to *Escherichia coli* and of sheep to *Chlamydia* (Nockels 1979). Several investigators have demonstrated that circulating neutrophils, peritoneal macrophages, and pulmonary alveolar macrophages from Se-vitamin E-deficient animals have low amounts of glutathione peroxidase activity and decreased microbial ability (Serfass & Ganther 1975, 1976).

Recent evidence indicates that Se significantly affects the function of all components of the immune system. The development and expression of non-specific humoral, and cell-mediated responses are included (Kiremidjan-Schumacher & Stotzky 1987). A deficiency of Se has been shown to inhibit

- resistance to microbial and viral infections
- neutrophil function
- antibody production
- proliferation of T and B lymphocytes in response to mitogens
- cytodestruction by T lymphocytes and NK cells

Although work in this area is still in its infancy, the effects of Se on key immunological functions indicate that this trace element may be an immunological response modifier of great clinical significance through its ability to augment and/or restore effector mechanisms or mediators of host defense systems.

CONCLUDING REMARKS

Manifestations of vitamin E and selenium deficiency depend on the degree of deficiency, other dietary factors, and the activity of tissues at the time of the deficiency. Myopathy seems more prevalent in growing animals that are physically active. Liver necrosis is more often observed in pigs reared in confinement, although myopathy is also present in most cases. During reproduction-lactation, active and proliferating tissues such as the fetal

placenta, uterus, and mammary gland are more susceptible to injury and infection.

Most data indicate that a deficiency in Se results in a strong oxidizing micro-environment, which is probably caused by the reduced concentrations of glutathione peroxidase. This in turn can lead to auto-oxidative damages to cellular membranes and cytoskeletal structures by reactive oxygen species, and result in disturbance of cellular processes that depend on the integrity of cell membranes and associated structures.

In general, a deficiency in Se appears to result in immunosuppression, whereas supplementation with low doses of Se appears to result in augmentation and/or restoration of immunologic functions.

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10 Relations between selenium and immunity

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Selenium has been shown to have an immunostimulatory effect in a variety of species when administered to animals in a deficient and also a normal selenium status. The response to selenium supplementation appears to be independent of vitamin E nutrition. The paper gives a summary of the effects of selenium on the antibody production, lymphocyte functions, phagocyte activities and disease resistance.

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The nutritional status of the host can influence the immunological defence systems. Several nutrients have beneficial effects on both antigen specific and non specific immune response. The control of oxidative reactions is critical to the maintenance of many aspects of immune function. Although free radicals are required for certain immune activities (killing of infectious organisms), the overproduction of these highly reactive molecules can result in adverse effects. Oxidative damage to lipid components of leucocyte membranes initiates the process of prostaglandin and leucotriene synthesis leads to loss of fluidity as well as alteration of receptor functions.

There are several essential dietary constituents that can inactivate free radicals. Selenium (Se) is an essential component of glutathione peroxidase (GSH-Px). This enzyme is important in the decomposition of hydrogen peroxide and lipid peroxides, the cytotoxic termination products of free radical attack on lipids. Such mineral antioxidant enzymes are primarily intracellular, therefore extracellular free radicals, either endogenously produced or from the environment, must be inactivated by the circulating antioxidants such as the direct acting vitamins. Vitamin E is present in all cellular membranes and protects against lipid peroxidation.

SELENIUM AND ANTIBODY PRODUCTION

Several studies have been conducted on the effect of Se on the antibody production in animals and chickens with Se- and/or vitamin E deficiencies (Tables 1 and 2, Mulhern et al. 1985). Marsh et al. (1981) (Table 1) demonstrated that two-week-old chicks maintained on Se- or Se-vitamin E-deficient diets from hatching had reduced antibody titres to sheep

red blood cells (SRBC), whereas in three-week-old chicks only the Se-vitamin E-deficient group was disadvantaged. This indicates an age-dependent effect of Se on the antibody production. The same group (Marsh et al. 1986) showed in a later work that Se was of importance for the development of the bursa of Fabricius and the thymus. In chicks maintained on diets deficient in Se and/or vitamin E from hatching, single deficiencies impaired the bursal growth, whereas a similar effect on the thymus was only seen in Se-vitamin E-deficient animals. Reduced numbers of lymphocytes resulting from the combined Se-vitamin E deficiencies were seen in both the thymus and bursa.

Table 1. The effects of selenium and/or vitamin E (supply or adequate diet) on the humoral immune response in chicken and animals compared with control chicken/animals fed a low selenium/vitamin E diet

Species	Se/E supply	Immunological effects	References
Chicken		Low Se: ↓ AB to RBC (2 weeks) Low E: ↓ AB to RBC (2 W) Low Se/E: ↓ AB to RBC (2 W) ↓ (3 W)	Marsh et al. 1981
Mouse	Se 5μg	↑ Primary AB to RBC	Spallholz et al. 1975
Mouse	Se 0.25-9.5 ppm	↑ AB (IgG) to RBC ↑ AB (IgM) to RBC Optimal supply 1.75 - 2.25 ppm	Spallholz et al. 1973
Horse	Se 5 mg/d E 600 mg/d Se/E 5mg/600mg/d	High E: ↑ AB to tetanus and to EIV High Se/E: ↑ AB to tetanus and to EIV	Baalsrud & Øvernes 1986
Horse	Se 0.22 ppm	↑ Primary AB to RBC ↑ IgG	Knight & Tyznik 1990
Pig	Se 5 mg	↑ IgM (colostrum) ↑ IgM, IgG (piglets)	Hayek et al. 1989

↑ increase, → no effect, ↓ decrease, AB antibody titre, EIV equine influenza virus, Ig immunoglobulin, RBC red blood cell

In both single-stomach mammals (Table 1) and ruminants (Table 2) the primary antibody response and the total IgM concentration were particularly influenced by Se deficiency. However, some studies demonstrated also that Se supplementation could increase serum IgG and the production of secondary antibodies to antigen. This indicates that the helper T-lymphocyte dependent class switch is also influenced by Se deficiency.

Table 2. The effects of selenium and/or vitamin E (supply or adequate diet) on the humoral immune response in ruminants compared with control animals fed a low selenium/vitamin E diet

Species	Se/E supply	Immunological effects	References
Cattle	Se 25 mg E 340IU	Se+/E+: ↑ AB (IgG) to *Pasteurella haemolytica* SE+ or E+: → AB to Past. h.	Droke & Loerch 1989
Calves	Se 80-200 ppm mineral mix.	↑ AB to egg lysozyme	Swecker et al. 1989
Calves	Se 30 mg /60 days	↑ IgM, → IgG by stress ↓ AB to Past.h.	Stabel et al. 1989
Calves	Se 0.2 ppm	↑ IgM, → IgG ↑ AB to IBRV	Reffett et al. 1988a
Lambs	Pellet Se	↑ AB to *Leptospira* → AB to *Brucella* → AB to C.pseudotbc.	Ellis et al. 1990
Sheep	Pellet Se	↑ Primary AB to *Brucella abortus* (↑) AB to RBC → AB to C.pseudotbc.	Jelinek et al. 1988
Lambs	Se 0.2 ppm	↑ IgM ↑ Primary AB to PIV-3	Reffett et al. 1988b
Lambs	Se 0.1-1.0 ppm	(↑) IgG (↑) AB to tetanus tox. PIV-3, C. pseudotbc.	Larsen et al. 1988a
Sheep	Se 100mg[1] E 50mg	Se+: ↑ AB to tetanus toxoid E+: ↑ AB to tetanus toxoid Se+/E+: no additive effect	Larsen et al. 1988a

↑ increase, → no effect, ↓ decrease, AB antibody titre, EIV equine influenza virus, Ig immunoglobulin, RBC red blood cell, IBRV infectious bovine rhinotracheitis virus, PIV-3 parainfluenza virus-3, C. pseudotbc. *Corynebacterium pseudotuberculosis*, Past. h. *Pasteurella haemolytica*

[1] As barium selenate

The immunological effects of Se supplementation on the antibody responses depend on both age and antigen (Table 2). In addition, dietary Se concentrations (1 mg/kg sodium selenite) which gave the best antibody response to tetanus toxoid in lambs were less effective than 0.1 mg Se/kg (sodium selenite) in their response to parainfluenza-3 virus and *Corynebacterium pseudotuberculosis* (Larsen et al. 1988a). The supplementation of both Se and vitamin E was necessary in some species (cattle and horse) in order to increase the antibody response to *Pasteurella haemolytica*, tetanus toxoid or influenza virus (Tables 1 and 2), whereas no additive effect of Se and vitamin E was found in lambs.

SELENIUM AND LYMPHOCYTE FUNCTIONS

Dietary deficiencies of the antioxidant micronutrients may affect T lymphocytes to a greater degree than B lymphocytes. T-cell membranes are more fluid than B cell membranes and T-cell lipids are more susceptible to peroxidation than B cell lipids. Phytolectins, such as concanavalin A (Con A), phytohaemagglutinin (PHA) or poke weed mitogen (PWM), are commonly used as mitogens to assay the proliferative response of lymphocytes, whereby PHA and Con A stimulate T cells and PWM stimulates both T and B cells. Se deficiency alone or in combination with vitamin E depresses the lymphocyte responses to mitogens and mixed lymphocyte responses (Tables 3, 4 and 8, Marsh et al. 1987). Where only Se is deficient, responses to PHA generally become more depressed than the responses to PWM. This is supported by the work of Eskew et al. (1985) where the responses of lymphocytes to the B-cell mitogen *Escherichia coli* lipopolysaccharide (LPS) were depressed in the Se-vitamin E-deficient rats, but not in Se-deficient rats.

Table 3. The effects of selenium and/or vitamin E (supply or adequate diet) on the lymphocyte response in ruminants compared with control animals fed a low selenium/vitamin E diet

Species	Se/E supply	Immunological effects	References
Lambs	Se 0.1-0.5 ppm	↑ Lymphocyte response to PWM, PHA, CON A	Larsen et al. 1988b
Sheep	Se 0.1-0.5 E 50mg	Se+: ↑ response to PHA → PWM, CON A E+: → response to PHA,PWM,CON A Se+/E+: ↑ response to PHA, PWM	Larsen et al. 1988b
Lambs		Low Se: ↓ lymphocyte response to PHA	Turner et al. 1986
Lambs		High Se: (↑) lymphocyte response to PHA, PWM Effect of serum (Se+) *In vitro*	Turner et al. 1985
Goat		Low Se: ↓ lymphokin (MIF), → IL-2, → lymphocyte response to CON A	Aziz & Klesius 1985
Cattle	Se 0.35 ppm	Low Se: ↑ TXB_2, $PGF_{1\alpha}$ ↑ PGE_2, LTB_4	Maddox et al. 1991

↑ increase, → no effect, ↓ decrease, IL-2 interleukin-2, PG prostaglandin, TXB thromboxane, LTB leucotriene polyclonal mitogens: PHA phytohaemagglutinin, PWM poke weed mitogen, CON A concanavalin A MIF migration inhibition factor

Table 4. The effects of selenium and/or vitamin E (supply or adequate diet) on the lymphocyte response in animals and chicken compared with control animals/chicken fed a low selenium/vitamin E diet

Species	Se/E supply	Immunological effects	References
Pig	Se/E 0.2ppm/ 311 IU/kg	Low Se/E: ↓ lymphocyte response to PHA, CON A, PWM serum: mitogen suppressive → NK activity, ADCC	Lessard et al. 1991
Pig	Se 0.5ppm /E 100ppm /riboflavin 5ppm	↑ T-cell number, response to PHA ↑ lymphocyte response to horse Ig (↑)AB to horse Ig	Rafai et al. 1989
Pig	Se 0.05-0.1 ppm	↑ Lymphocyte response to PHA	Larsen & Tollersrud 1981
Chicken		Low Se/E: ↓ response to CON A Low Se: ↓ response to CON A	Marsh et al. 1986
Mouse	Se 0.02-0.2-2.0 ppm	High Se: ↑ tumour kill Low Se: ↓ T_c-activity ↓ lymphotoxin	Roy et al. 1990
Mouse	Se 0.02-0.2-2.0 ppm	High Se: ↑ MLR, ↑ response to PHA Low Se: ↓ MLR, ↓ response to PHA High/low Se: → IL-2, IL-1	Kiremidjian-Schumacher et al. 1990
Rat	Se 0.5-2.0-5.0 ppm	0.5/2 ppm: ↑ NK-Activity 0.5-5.0 ppm: ↓ DTH response, → IL-1 5.0 ppm: ↓ AB to KLH, ↓ PGE_2	Koller et al. 1986

↑ increase, → no effect, ↓ decrease, AB antibody titre, IL-1 interleukin-1, IL-2 interleukin-2, T_c T cytotoxic cell, MLR mixed lymphocyte response, DTH delayed type hypersensitivity, PGE prostaglandin, KLH keyhole limpet haemocyanin, Ig immunoglobulin, polyclonal mitogens: PHA phytohaemagglutinin, PWM poke weed mitogen, CON A concanavalin A, NK natural killer cell, ADCC antibody-dependent cell-mediated cytotoxicity

Adult ruminants appear to be more resistant to the immunological effects of a Se deficiency on the lymphocyte proliferation (Turner & Finch 1991). Ewes maintained on rations low in both Se and vitamin E showed good responses to PHA, whereas cells taken from the lambs all showed a decreased T-cell reactivity (Turner & Finch 1990). They explained these observations with increased availability of rumen microbial Se to the lymphocytes (Turner & Finch 1991).

The effector part of the T-cell response to antigens comprises both generation of effector lymphocytes and activation of phagocytes. The mitogen-induced production of leucocyte migration inhibition factor was inhibited by lymphocytes from Se-deficient goats

in comparison with Se-adequate animals, although the interleukin-2 production and the lymphocyte response to Con A were not influenced (Table 3: Aziz & Klesius 1985). Reduced production of migration inhibition factors may also have an effect on conditions with chronic inflammation, which is associated with macrophage infiltration and activation. In mice maintained on diets low in Se, Roy et al. (1990) found reduced NK and T-cell-mediated cytotoxic activity accompanied by decreased production of lymphotoxin, and they also found that Se supplementation increased the generation of cytotoxic lymphocytes and the ability to destroy tumour cells (Table 4).

The possible association of Se deficiency with reduced cell-mediated immunity may be caused by down regulation of interleukin-2 (IL-2) receptor expression, which thereby limits IL-2-mediated cellular immune responses. The ability of cells from Se-deficient mice to proliferate in response to PHA and to allogeneic stimulation in the mixed lymphocyte reaction, was inhibited compared with that of cells from Se-adequate mice (Kiremidjian-Schumacher et al. 1990). Both responses, however, were increased by Se supplementation. The observed effects of Se on the lymphocyte responsiveness were independent of the IL-1 or IL-2 production, but it was suggested that Se may have modulated the expression of IL-2 receptors on the cell surface, which could explain the altered ability of lymphocytes from Se-deficient animals to respond to mitogens and antigens. Other studies indicated also that changes in the proliferative capacity of lymphocytes according to Se status were not due to an altered ability of macrophages to produce IL-1 or to present antigens (Eskew et al. 1985: Koller et al. 1986).

Although the production of IL-1 is not influenced by the Se status, altered secretion of arachidonic acid metabolites by macrophages could have important regulatory implications. In cattle, Maddox et al. (1991) (Table 3) demonstrated that there were marked effects of dietary Se on milk eicosanoid concentrations in response to an *E. coli* infection. Thus, increased concentrations of thromboxane B_2, prostaglandin $F_{1\alpha}$, prostaglandin E_2 and leucotriene B_4 were associated with significantly greater numbers of bacteria in the milk probably because of decreased killing of *E. coli* by neutrophils from Se-deficient animals. Increased inflammation and tissue damage, caused by endotoxin and inflammatory mediators, will induce a higher tissue hydroperoxide concentration that, in a state of Se-deficiency, may lead to higher activity of cyclooxygenase and lipoxygenase enzymes, which increases the eicosanoid levels.

IN VITRO STUDIES

Cell culture conditions are influenced by the production of reactive oxygen intermediates. The oxidative products of cultured cells are not usually removed during the culture periods. Free radical mediated reactions result in membrane lipid peroxidation, which significantly depresses *in vitro* responses to mitogens as well as primary antibody responses. Addition of antioxidants to lymphocyte cultures may overcome the immunosuppression (Table 5). In ruminants the addition of sodium selenite to the cells in culture can increase the lymphocyte response to mitogens and the PWM-induced immunoglobulin production by the addition of serum or pooled plasma from animals with high Se status (Turner et al. 1985; Finch & Turner 1989; Stabel et al. 1990; Stabel et al. 1991). These findings indicate that the failure resides in the microenvironment of the cells rather than in the cells themselves.

Table 5. The effects of *in vitro* selenium or vitamin E supply on the lymphocyte response of ruminants and humans

Species	Optimal dose	Immunological effects	References
Sheep	Se:1-10 ng/ml: E:0.15-1.5μg/ml:	↑ Response to PHA, CON A, PWM ↑ Response to PHA, CON A, PWM E and Se differential influence	Finch & Turner 1989
Cattle	Selenite 100ng/ml Se.METH 100 ng/ml Se.CYS. 100 ng/ml	↑ Ig-Synthesis (PWM) Se.METH/Se.CYS. > selenite	Stabel et al. 1991
Cattle	Se 50-400 ng/ml Se 1600-3200 ng/ml	↑ Response to PWM, → PHA, CON A ↓ Response to mitogens	Stabel et al. 1990
Human	Selenite 0.1 μg/ml 0.5 - 1.0 μg/ml	↑ Neutrophil-cytotoxic activity ↑ NK-cytotoxic activity ↓ Cytotoxic activity	LaBue et al. 1988
Human	Se 10^{-3}-10^{-9} M	Low Se: ↑ IgG (PWM) Selenite > Se.METH./Se.CYS. High Se: ↓ IgG, IgM (PWM)	Reinhold et al. 1989

↑ increase, → no effect, ↓ decrease, Ig immunoglobulin,
polyclonal mitogens: PHA phytohaemagglutinin, PWM poke weed mitogen, CON A concanavalin A.
Se.METH. selenomethionine, Se.CYS. selenocystine, NK natural killer cell

The amount of Se needed (*in vitro*) to enhance the response of lamb lymphocytes to PHA in culture was best in the range 1-10 ng Se/ml, whereby toxic effects were evident beyond 1 μg/ml (Finch & Turner 1989). Similar findings were also demonstrated in cattle (Table 5). Sodium selenite or selenomethionine added to the diet at 0.1 or 0.5 μg/g enhanced the lymphocyte responses of lambs to mitogens whereas 1 μg/g (selenomethionine) had an inhibitory effect (Larsen et al. 1988b). Immunostimulatory effects of Se supplementation on the IgM and IgG antibody responses, T-cell cytotoxic activity, natural killer cell activity and mixed lymphocyte response were, however, found at considerably higher Se-concentrations in rodents than in sheep (Spallholz et al. 1973; Table 4: Roy et al. 1990; Kiremidjian-Schumacher et al. 1990; Koller et al. 1986).

SELENIUM AND PHAGOCYTE FUNCTIONS

Phagocyte-derived free radicals are essential for killing bacterial pathogens. The killing process is usually confined to intracellular vacuoles which enclose the phagocytosed pathogen. However, the reactive molecules leak from phagolysosomes into the surrounding cytoplasm and are also released into the intracellular spaces. Failure to detoxify these products could damage the cell's microbicidal and metabolic functions. Neutrophils contain superoxide dismutase, catalase and glutathione/glutathione peroxidase to protect against

auto-oxidation, but, in an antioxidant-deficient condition, they may be damaged by the reactive oxygen species.

Dietary deficiencies of Se and/or vitamin E have been shown to impair neutrophil and macrophage activities in humans (Table 5), chickens and animals (Table 6). The Se deficiencies are, however, not associated with reduced phagocytosis (Table 6: Grasso et al. 1990; Hogan et al. 1990; Boyne et al. 1986), but may be more likely in combined Se and vitamin E-deficient animals (Turner & Finch 1990) or chickens (Table 6: Diertert et al. 1990). The intracellular killing of yeasts and bacteria by neutrophils and macrophages from animals and chickens with Se deficiency is reduced (Table 6). These impaired functional properties may be restored by incubation with sodium selenite *in vitro* (Urban & Jarstrand 1986; Table 6: Aziz et al. 1984; Table 5: LaBue et al. 1988).

Table 6. The effects of selenium and/or vitamin E (supply or adequate diet) on the phagocyte function in chicken and animals compared with control chicken/animals fed a low selenium/vitamin E diet

Species	Se/Vit.E supply	Immunological effects	References
Cattle	Se 2 mg /day	→ Phagocytic activity ↑ Intracell. killing ↑ Viability (Staph.aur.) → Superoxide anion production ↑ Extracellular hydrogen peroxide	Grasso et al. 1990
Cattle	Se 50mg or E 680IU	E+:↑ Intracellular kill. OF Staph.aur, E.coli by neutrophils Se+:↑ kill of Stapf.aur. Se+/E+: no additive effect Suppl.:→ Phagocytosis	Hogan et al. 1990
Goat		Low Se: ↓ Neutrophil functions	Aziz et al. 1984
Chicken	LOW Se/E	↓ Peritoneal macrophages ↓ Viability ↓ Phagocytosis of RBC	Dietert et al. 1990
Rat		Se deficiency: ↓ kill of candida by neutrophils/macrophages and ↑ mortality Following Stapf.aur. injection	Boyne et al. 1986

↑ increase, → no effect, ↓ decrease, AB antibody titre, RBC red blood cells, Staph.aur. *Staphylococcus aureus*, E.coli *Escherichia coli*

SELENIUM AND DISEASE RESISTANCE

Although several studies show effects of Se supplementation on phagocyte and lymphocyte function, only a few studies demonstrate the effect of Se on the disease resistance in ani-

mals. There is, however, some indication that Se supplementation is beneficial for bacterial infections in animals and chickens (Table 7) and in laboratory rodents (for reference see Turner & Finch 1991), but there are only a few reports on increased resistance to viral infection. In children with Down's syndrome, which is a condition associated with susceptibility particularly to bacterial infections, Se increased the serum concentrations of IgG_2 and IgG_4, but not of IgG_1 and IgG_3 (Table 8: Anneren et al. 1990). In humans IgG_2 plays a role in the immune response to bacterial antigens, whereas viral infections trigger mainly IgG_1 and IgG_3 responses.

Table 7. The effects of selenium and/or vitamin E (supply or adequate diet) on the disease resistance in chicken and animals compared with control chicken/animals fed a low selenium/vitamin E diet

SpecieS	Se/VIT.E Supply	Disease resistance	References
Cattle	Se 0.35 ppm	↓ Mastitis ↑ Milk bacteria	Maddox et al. 1991
Cattle	Se 0.1mg/kg body weight E 320mg/d	E+: ↓ Mastitis 37% E+: ↓ Clin.sympt.44% Se+:↓ Clin.sympt.46% Se+/E+:↓ Clin.sympt.62%	Smith et al. 1984
Cattle	Se 0.1 mg/kg im.	↓ Metritis ↓ Cystic ov.	Harrison et al. 1984
Chicken	Se 0.25 ppm/ E 300ppm	↑ ND, ↑ AB to ND-virus	Bassiouni et al. 1990
Chicken	Se 0.25 ppm OR E 100IU	↑ *E.tenella* ↑ Weight ↑ *E.tenella* ↑ Weight	Colnago et al. 1984
Pig	Se 0.2-0.4 -0.8 mg	↑ *Treponema hyodysenteriae*	Teige et al. 1984

↑ increase, → no effect, ↓ decrease, AB antibody titre, ND Newcastle disease

Antioxidant supplementation enhanced phagocytosis and killing activity as well as lowering the circulating levels of lipid peroxides and immunosuppressive prostaglandins, perhaps the main reason for the effect of Se on mastitis and metritis (Table 7). Changes in the physical properties of membranes with ageing may influence the sensitivity of immune cells to antigenic stimuli. Immunosuppression in man associated with increasing age, early HIV infection and some clinical conditions was influenced by dietary Se (Table 8). Supplementation with Se enhanced the lymphocyte response to mitogens and to antigens in the elderly,

while a stimulatory effect of Se on IgG and IgA concentrations and the cytotoxic T-cell activity in humans with normal Se status could also be detected. These studies support the role of Se in the maintenance of an optimal immune response, and demonstrate the need for further investigation on the chemopreventive potential of these essential micronutrients.

Table 8. The effects of selenium supply on the immune response in human

Clinical conditions	Selenium supply	Immunological effects	References
Early HIV infection		Low plasma Se $<85\mu g/l$: ↓ NK-cytotoxic act. High Se $>120\mu g/l$: ↓ IgG, IgM, ↓ Lymphocyte response to PHA	Mantero-Atienza et al. 1991
Down's syndrome	1 year suppl.	↑ IgG_2,IgG_4, → IgG_1, IgG_3	Anneren et al. 1990
Short-bowel syndrome	$200\mu g/d$	↑ Lymphocyte response to Candida, varicella but → low to tetanus toxoid ↑ T-cell number (CD3) ↑ Lymphocyte response to PHA, PWM	Peretz et al. 1991a
Elderly	$100\mu g/d$	↑ Lymphocyte response to PWM	Peretz et al. 1991b
Normal	$100\mu g/d$	↑ IgG, IgA and ↑ T_C → IgM, → T cells, B cells	Herzfeld et al. 1989
Elderly	$100\mu g/d$	↑ Lymphocyte response to mitogens Suppressor factors in serum	Peretz 1990
Marginal Se	$200\mu g/d$	↑ Lymphocyte response to mitogens and to antigens	

↑ increase, → no effect, ↓ decrease, AB antibody titre,
polyclonal mitogens: PHA phytohaemagglutinin, PWM poke weed mitogen
T_c T cytotoxic cell

CONCLUSION

Further studies are needed to elucidate the immunoregulatory effect of selenium and to test the effect on different clinical conditions to investigate whether it can be of clinical value in the management of infections. Identification of the immunologically active form of Se supplementation for the immunocompetence should be tested and correlated with resistance to infections. Clinical trials on disease resistance can be supported with *in vitro* assays if one includes immunological parameters which are believed to be closely related to protection against a particular disease. However, the microbiological environment, the anti-oxidant micronutrient status as well as physiological parameters should be properly controlled.

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11 Optimum selenium levels in animal products for human consumption

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Reported or suggested health effects resulting from geographical variations in the selenium intake in different regions of the world are discussed. Deficient selenium intake may be associated with cardiomyopathy and/or osteoarthrosis. Extremely high selenium intake is associated with hair loss, nail changes and in some cases even neurological signs and symptoms. A daily intake of 50-400 μg selenium has been suggested to constitute a safe and adequate zone. Recent case-control studies indicate, however, that some anticarcinogenic properties of selenium may operate also in the dose range of 50-70 μg Se/day, and an optimum intake zone of 70-400 μg/day is suggested here. Furthermore, to ensure an adequate daily Se intake, a contribution from animal products of at least 30 μg/day is proposed. In low selenium areas, such as Scandinavia, the intake from meat and other animal products is often less than 20 μg/day. Supplementation of animal feed with inorganic selenium compounds has raised the Norwegian Se intake by 5-10 μg daily, but the contribution of Se from animal products still lies at the lower margin of the recommended zone.

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For selenium (Se) as for other essential elements, there exists a zone of safe and adequate daily intake. Extremely high or extremely low doses of the element will give rise to impaired performance or overt illness. At both ends of the safe and adequate zone there are marginal or critical levels that may involve health hazards under certain circumstances, but these intake levels may be difficult to identify (see Fig. 1).

There are defined regions in the world, characterized by very low or very high daily selenium intakes. Blood serum levels are often used as an indicator of the dietary Se intake, and data from various reports (Fig. 2) support the usefulness of this indicator, at least when daily intakes are lower than 100 μg/day. At intakes exceeding 100 μg/day, the whole blood selenium appears to be more linearly related to the amount of selenium intake (Aaseth & Thomassen 1988).

When discussing optimum selenium levels in animal products for human consumption, it is crucial to identify the safe and adequate intake zone, and then discuss a recommended fraction derived from animal products.

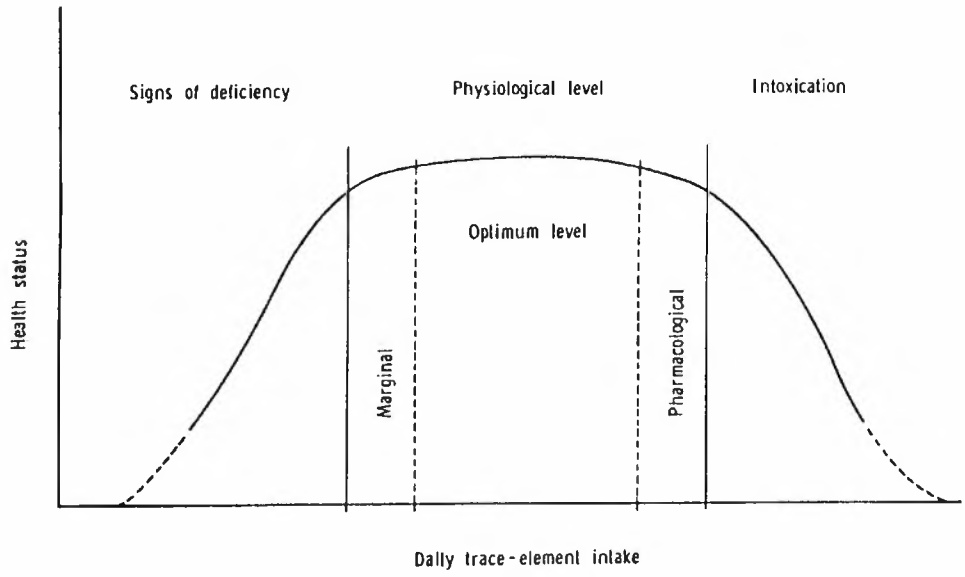

Fig. 1. Health status as a function of trace element intake

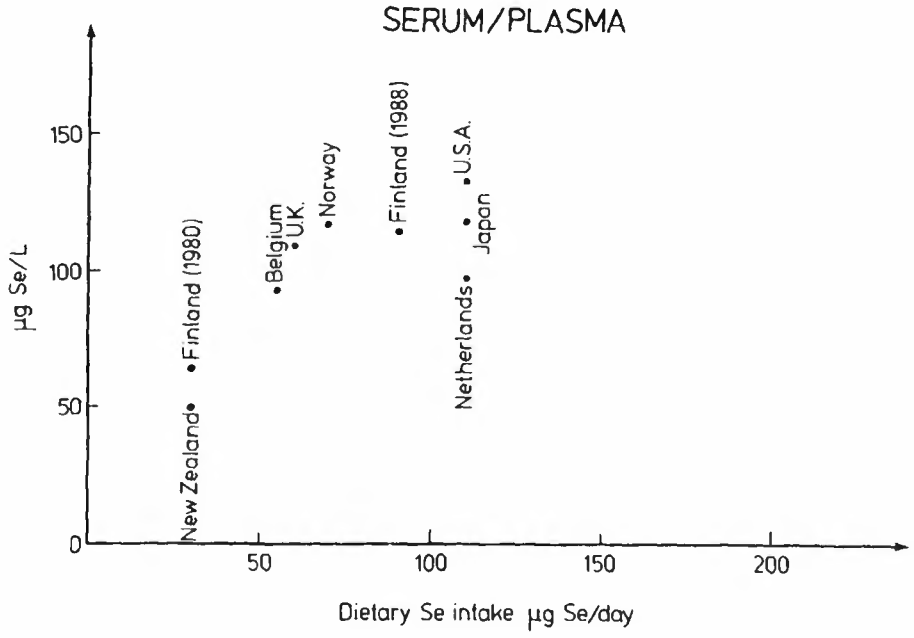

Fig. 2. The relationship between the dietary selenium intake and concentration found in serum/plasma

MARGINAL OR DEFICIENT SELENIUM INTAKE

Experiences from low-selenium areas in China have shown that a daily selenium intake as low as 20 μg involves a health hazard for the development of a cardiomyopathy, referred to as *Keshan disease*. This cardiac disease is characterized by multifocal necrosis and fibrous replacement of the muscle cells. Geographically, the cases presenting the syndrome have been localized within a belt-like zone reaching from the north-eastern to the south-western part of China. Average whole blood selenium of individuals in the affected areas was as low as about 20 μg/L, and average Se intake per day was 20 μg or lower (Yang et al. 1984). Keshan disease is purported to have a multifactorial aetiology, with Se defiency rendering the individuals less resistant to cardiotoxic agents. While efficient prophylaxis is obtained by Se supplementation, such treatment does not cure the overt cases.

Another syndrome reported from the selenium deficient areas is *Kashin-Beck disease*, which is an endemic osteoarthrosis.

Unlike the well-known polyarthrosis affecting elderly people in western countries, this clinical entity usually affects children. And growth is inhibited, as the metaphyseal zones are involved in addition to the joints. In addition to the selenium defiency, a possible role of low vitamin E status, a mycotoxin contamination, or other environmental factors have been discussed (Yang 1987). However, selenium supplementation has both prophylactic and therapeutic efficiency.

A possible relationship between low selenium and *increased cancer risk* is under investigation in Chinese regions (Yu et al. 1985), and has been studied in several case-control studies with a prospective approach in western countries (see Fig. 3): one study from the Boston region of the United States (Willett et al. 1983), another from Finland by Salonen et al. (1984) and two from Norway (Ringstad et al. 1988; Glattre et al. 1989). All of these four studies have used a follow-up period of about five years between prediagnostic serum selenium determination and the cancer diagnostics. Adequately matched controls have been chosen from some populations as the cases. In all four studies the cases appeared to have a prediagnostic serum selenium value about 5-10% lower than the control values. Further analyses of the studies from the United States and from Finland revealed that the differences between cases and controls were significant for smokers, but not for non-smokers. All the above studies are compatible with the hypothesis precipitated from early animal experiments, that selenium in some unexplained way contributes to the defence mechanisms of the mammalian body against environmental carcinogenic agents. The dose-response relationship for this suggested anticarcinogenic action of selenium is not yet known. Although the selenium intake in the control group in the study from Finland was as low as about 30 μg/day, the estimated intakes in the studies from Norway and from the Boston region were 70-100 μg/day, indicating that some anticarcinogenic properties of selenium may operate at daily doses up to at least 70 μg.

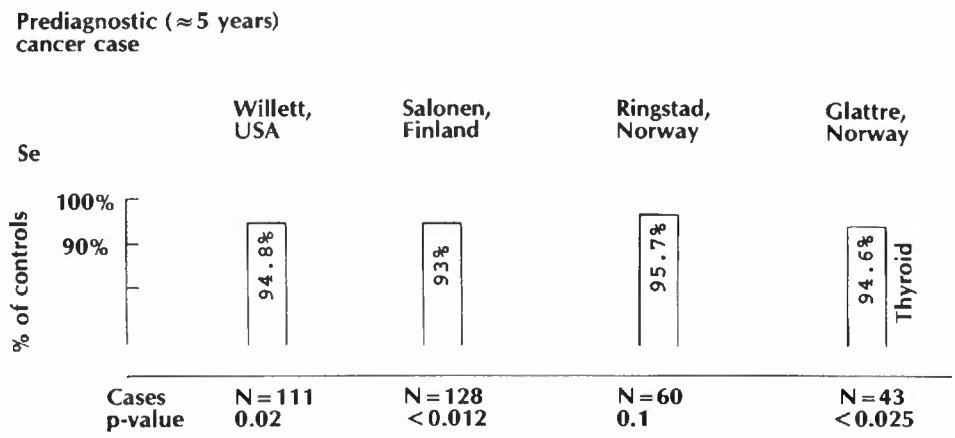

Fig. 3. Results of case-control studies on serum selenium increased cancer risk in the United States (Willett et al. 1983), in Finland (Salonen et al. 1984) and two studies from Norway (Ringstad et al. 1988; Glattre et al. 1989), the latter Norwegian study being focused exclusively on thyroid cancer. In all four studies the serum selenium values are determined about five years before the cancer diagnosis, and the mean values of cases are given in percent of control value (see text). The number of cases, as well as the calculated p-values are also given in the figure

CRITICAL AND TOXIC SELENIUM DOSES

Recently, we carried out a selenium supplementation trial, double-blinded with a placebo group, in Norwegian patients suffering from arthritis (Aaseth & Weberg Teigen 1993). We used relatively high doses of selenomethionine, 600 μg Se/day, over a period of 8 months.

Serum values reached a plateau of about 500 μg/L, whereas whole blood selenium rose to about 680 μg/L. No overt signs of toxicity were observed, clinically or biochemically. However, the supplemented group obtained a slight reduction in the rheumatoid factor titre, which is consistent with the assumption that subtoxic (pharmacological) selenium doses may possess immunomodulating properties (Aaseth & Weberg Teigen 1993).

The problem of overt selenium intoxication has been reported from Enshi county in southern China, in the period 1961-64 (Yang 1987). The most common symptoms were hair loss and diseased nails. Some neurological symptoms were also recorded. The source of the excessive intake was vegetable food grown in an extremely seleniferous area. Average daily intake in the population with endemic cases was almost 5,000 μg Se/day. In another seleniferous area with an average daily intake of about 750 μg the inhibitants did not present symptoms of intoxication (Yang 1988).

RECOMMENDATIONS

Responsible recommendations for selenium intake involve considerations of the selenium status in terms of both its nutritional essentiality as well as its toxic potential. In the derivation of values encompassing a zone of safe and adequate intake, possible uncertainties in the reported observations must be taken into account. It appears from the discussion above that health hazards are associated with intakes as low as 20 μg/day, as well as with intakes exceeding 750 μg/day. A narrowing of the safe and adequate zone to 50-400 μg Se/day is recommended.

Furthermore, it has been suggested that the lower limit of the optimum zone might be somewhat higher than 50 μg/day, at least if the suggestions and reports on anticarcinogenic properties of selenium hold true. Thus, the recommended dietary allowances (RDA) estimated by the Food and Nutrition Board of the National Research Council of the United States are 70 μg/day for men and 55 μg/day for women (see Combs 1992).

Based upon the discussion in the present paper, it is tempting to propose 70-400 μg/day as a safe and adequate zone. A relatively safe recommendation to obtain this is that animal products for human consumption (meat and diary products) should make up at least 30 μg Se daily.

Scandinavia is known to be a low-selenium area, which means that Scandinavian meat, as well as the grain, contains very low levels of selenium. In Norway, the average selenium intake has been acceptable, owing to the extensive use of grain imported from high-selenium areas in northern America.

However, meat accounts for less than 20 μg Se/day in the usual Norwegian diet, and supplementation of animal feed with selenite compounds has resulted in an average increase in daily selenium intake of less than 10 μg (Frøslie et al. 1985). The addition of selenium in commercial fertilizers gives rise to higher selenium accumulation in meat and dairy products, according to experiences from Finland (Sippola & Jansson 1991), as the domestic livestock will then be exposed to organic selenium from the vegetation. A recommended intake of selenium from animal products above 30 μg/day appears to be more easily obtained by supplementing the animal feed with organic selenium compounds, than with selenite in low selenium areas. However, more research is required, particularly on the possible relationships between the various forms of cancer disease and a low or deficient selenium intake, before more precise recommendations of optimum selenium levels in animal products can be given.

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12 Disorders related to selenium deficiency in man

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Severe selenium deficiency due to low intakes (< 10 μg per day) has been shown to cause cardiomyopathy, known as Keshan disease, in the People's Republic of China. The relationship between selenium status and other cardiovascular diseases is less clear, but in Finland low serum selenium concentrations in the 1970s were found to be associated with increased risk of cardiovascular death in two prospective case-control studies. At higher levels there seems to be no correlation between serum selenium and the development of cardiovascular diseases. With respect to cancer, an inverse association has been found in several prospective case-control studies between prediagnostic serum selenium concentration and the risk of cancer. Most significant associations have concerned cancer of the upper gastrointestinal tract and of the lung, particularly in men. In many studies no significant associations have been found and the association between serum selenium and the risk of subsequent breast cancer seems definitely non-significant. Recently, it has been shown that the peripheral deiodination of thyroxine is regulated by a selenoenzyme. Selenium deficiency and its rapid correction may affect thyroid function and aggravate hypothyroidism in subjects who have concomitant iodine deficiency. Although severe selenium deficiency may have definite harmful effects, it it still unclear whether mild selenium deficiency exerts important effects on human health.

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SELENIUM AND CARDIOVASCULAR DISEASES

Keshan disease

In the People's Republic of China there are areas of selenium deficiency and also of selenium excess. Dietary selenium intake is extremely low in a large sector extending from the southwest to the northeast of the country. In roughly the same areas an endemic, often fatal cardiomyopathy is found affecting particularly young children and women of childbearing age. This disorder is called Keshan disease and affects people whose serum selenium levels are below 10 μg/l, corresponding to intakes of around or below 10 μg per day. The condition can be prevented by prophylactic administration of sodium selenite (Chen et al. 1980). It is believed that selenium deficiency combined with a viral infection or with other dietary factors is responsible for the development of the disease but there is no direct evidence of this.

The findings in Keshan disease are characterized by multiple focal areas of myocardial necrosis without changes in the coronary arteries. There are occasional reports of patients receiving total parenteral nutrition showing low serum selenium concentrations and similar changes in the myocardium (Virtamo & Huttunen 1988).

Other cardiovascular diseases

There have been reports of geographical differences in the incidence of coronary heart disease (CHD) related to differences in mean selenium intake and reports on reduced serum selenium concentrations in patients with diagnosed CHD or congestive cardiomyopathy (Virtamo & Huttunen 1988). In cross-sectional studies the changes in serum selenium may be secondary effects caused by the disease. More reliable information is provided by the examination of the correlations between prediagnostic serum selenium concentrations and the incidence of cardiovascular diseases in prospective case-control studies.

There are seven published studies with follow-up periods of 5-10 years (Virtamo & Huttunen 1988, 1991) and a recent study with a three-year follow-up from Denmark (Suadicani et al. 1992). Four of the studies are from Finland where low mean selenium intakes in the range of 25-30 μg/d were found in the 1970s, attributable a low selenium content in domestic foods (Varo & Koivistoinen 1980). In the study carried out by Salonen et al. (1982), cardiovascular events were recorded during a follow-up period of seven years in a cohort of 11,000 persons from eastern Finland. Serum selenium concentrations of 283 persons who had an acute myocardial infarction or who had died of cardiovascular disease and concentrations of a similar number of persons who had remained free of cardiovascular disease during the follow-up period were determined from samples obtained ten years earlier. An increased relative risk of cardiovascular and coronary deaths and of myocardial infarction was observed in persons belonging to the lowest tertile of serum selenium concentrations with values of less than 45 μg/l at the beginning of the study. Serum selenium concentrations below 35 μg/l reflecting very low intakes around or below 20 μg/d were associated with the highest risk of cardiovascular death. The mean serum selenium concentration of the control subjects was 55.3 μg/l.

In the study by Virtamo et al. (1985) on a five-year follow-up of men from eastern and southwestern Finland an increased risk of borderline statistical significance was found for cardiovascular deaths and stroke deaths but not for coronary heart disease for subjects with low serum selenium levels. The other Finnish studies failed to show a significant association between prediagnostic serum selenium concentration and the incidence of coronary heart disease (Miettinen et al. 1983; Salonen et al. 1985b). In the study by Miettinen et al. (1983), which included men belonging to the upper social class, none of the subjects had a serum selenium concentration below 50 μg/l. In accordance with this, both the Dutch study by Kok et al (1987b) and the studies by Ringstad & Thelle (1986) and Ringstad et al. (1987) from northern Norway in subjects with mean serum selenium concentrations around 100 μg/l, showed no associations between serum selenium and the risk of coronary heart disease or cardiovascular deaths. Suadicani et al. (1992) from Denmark reported that male subjects whose serum selenium concentration was equal to or less than 1 μmol/l (< 80 μg/l), representing the lowest tertile of the distribution in Denmark, had an increased risk of ischemic heart disease (IHD) during a three-year follow-up. These men also displayed an increased all-cause mortality from causes other than IHD.

In general, there have been no clear associations between serum selenium and the known risk factors of coronary heart disease (Bukkens et al. 1990), but Salonen et al. (1988) reported on a significant negative association between risk-factor-adjusted secondary platelet aggregation and serum selenium level in men from eastern Finland. This preliminary finding has not been confirmed elsewhere.

SELENIUM AND CANCER

Numerous experimental studies in animals have indicated that selenium supplementation in high doses decreases the incidence of cancer (Ip 1985). These studies and early findings suggesting geographical differences in cancer mortality in relation to exposure to selenium in food have stimulated research in humans. In cross-sectional case-control studies patients with diagnosed cancer have generally shown lower serum selenium levels than healthy control subjects (Willett et al. 1991). The secondary effects of malignant disease on food intake and metabolism cannot be separated from the possible primary effect of a low serum selenium concentration in these studies. Therefore it is more useful to look at the results of prospective studies in which the samples for selenium analysis have been collected before the onset of clinical disease.

In the first prospective study of selenium and cancer, serum samples from a multicenter hypertension trial in the USA were analyzed (Willett et al. 1983). The mean serum selenium concentration of the 111 subjects who developed cancer during the subsequent five years was lower (129 ± 2 μg/l, mean$\pm$SEM) than that of 210 control subjects who remained free of cancer (136 ± 2 μg/l, $p=0.02$). The excess risk was most pronounced in the lowest quintile of serum selenium values (< 115 μg/l). In a similar study from Finland (Salonen et al. 1985a) the subjects who developed cancer also had lower mean serum selenium values (50.5 ± 1.1 μg/l) than those of matched control subjects without cancer (54.3 ± 1.0 μg/l, $p=0.01$). Most of the excess risk was in the lowest third of the serum selenium distribution (<45 μg/l). Thus, a similar association between serum selenium levels and the risk of cancer was found in two populations with totally different distributions of serum selenium concentrations. It is apparent from these and from subsequent studies that all cancers do not show similar associations with selenium. As the number of cases who developed cancer has been rather small in most studies, it is difficult to draw reliable statistical conclusions concerning cancer in different locations (Willett et al. 1991).

The largest reported material comprises the ten-year follow-up of the 39,268 subjects who participated in the health examination survey of the mobile clinic of the Finnish Social Insurance Institution in 1968-72 (Knekt et al. 1990). During the follow-up 1,096 new cases of cancer were identified, 597 in men and 499 in women. Selenium was determined from stored serum samples of each case and two control persons matched for gender, municipality and age. In males the mean serum selenium concentration of the cases (59.1 μg/l) was lower than that of the control subjects (62.5 μg/l, $p < 0.001$). In women the respective mean values were equal, 63.6 and 63.9 μg/l. In males the smoking-adjusted serum selenium values, divided into quintiles, showed a statistically significant inverse trend with the relative risk of cancer at all sites and with cancer of the stomach, pancreas, and

lung but not with colorectal cancer, cancer of the prostate or cancer of the urinary tract. In women no association was found between serum selenium quintiles and the relative risk of cancer.

In accordance with these findings, other studies in which the data have been analyzed separately for men and for women have failed to show a significant association between selenium and subsequent development of cancer in women (Willett et al. 1983; Salonen et al. 1984; Kok et al. 1987a; van den Brandt et al. 1993). Willett et al. (1991) reviewed the studies on the relationship between serum or toenail selenium concentrations and the risk of breast cancer and concluded that these studies comprised sufficient numbers of cases to exclude significant statistical association. Lower prediagnostic serum selenium values in persons who developed cancer as compared with subjects who remained free from cancer have been reported in additional studies from Finland (Salonen et al. 1985a), Sweden (Fex et al. 1987), Norway (Glattre et al. 1989) and the USA for cancer of the bladder (Helzlsouer et al. 1989) and cancer of the pancreas (Burney et al. 1989). In a Dutch study by van den Brandt et al. (1993) toenail selenium levels showed a significant inverse association with the risk of stomach cancer but not with the risk of colorectal cancer in men.

On the other hand, in several prospective studies from Finland (Virtamo et al. 1987), Norway (Ringstad et al. 1988) and the USA (Peleg et al. 1985; Menkes et al. 1986; Nomura et al. 1987; Schober et al. 1987; Coates et al. 1988) no statistically significant associations were observed between serum selenium and the risk of cancer at different sites. With the exception of the Finnish study, low selenium values have been unusual or absent in these studies.

SELENIUM AND THYROID HORMONES

In rats selenium deficiency decreases the activity of type I (peripheral) 5'-deiodinase, which has been found to be a selenoprotein (Arthur et al. 1990; Behne et al. 1990). This enzyme, which converts thyroxine (T_4) into triiodothyronine (T_3), contains selenocysteine (Berry et al. 1991).

In certain sectors of Zaire, where there is concomitant deficiency of iodine and selenium, endemic cretinism is characterized by the existence of hypothyroidism in addition to the common findings of mental and growth retardation. It has been hypothesized that in conditions where the production of hydrogen peroxide in the thyroid is stimulated by increased secretion of TSH, selenium deficiency, by reducing the activity of glutathione peroxidase, might damage the thyroid through toxic effects of excess hydrogen peroxide (Corvilain et al. 1993). Supplementation with selenium of hypothyroid subjects who have a combined deficiency of iodine and selenium reduces the serum T_4 concentration and might aggravate hypothyroidism (Contempré et al. 1991). Thus selenium deficiency seems to affect the metabolism of thyroid hormones even in man, although it is not known whether this is of clinical importance for people living in affluent countries.

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13 General aspects of selenium fertilization

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Selenium (Se) is unevenly distributed in the soils, which results in areas that are either Se-toxic or Se-deficient for the animals. There are a number of methods to secure an adequate Se supplementation for livestock in the Se-deficient areas. Among these are fertilization and foliar application of Se to crops. A number of factors influence the uptake of Se into the plants, but these effects have been well described. One advantage of these methods is the transformation in the plants of inorganic Se to bioavailable, organic Se compounds. Thus, fertilization and foliar application with Se are easy, cheap, and secure ways of raising the Se concentration in fodder crops to an adequate level.

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For more than 30 years selenium (Se) has been known as an essential trace element for animals and humans, and in many countries lack of Se in food is a common example of mineral imbalance caused by intensive plant production. Several attempts have been made to demonstrate the essentiallity for plants too, by depleting the growth medium of Se, but so far without success. Furthermore, of all the Se-containing biologically active components found in animals, none are found in plants. Consequently, the interest for Se in plants is related to the quality of the plants as the main source of selenium for humans and animals, and whether it is possible to affect the selenium nutrition of the livestock and people by field treatments.

GEOGRAPHICAL DISTRIBUTION OF Se

The minimum Se concentration of the total fodder that will meet animal requirements is considered to lie in the range of 0.05-0.1 ppm, and toxic effects can be expected at chronic intakes of fodder that exceed about 1 ppm Se. On the basis of these limits, different areas of the world are characterized as Se-deficient, Se-adequate, and Se-toxic areas. Outside Europe, crops containing toxic Se concentrations are found in the mid-western regions of the US and Canada, in Venezuela, India, and China, but Se deficiency areas are more widespread, and has been reported from both the western and the eastern coastal areas of North America, from Venezuela, Australia, New Zealand, Japan, and China (Gissel-

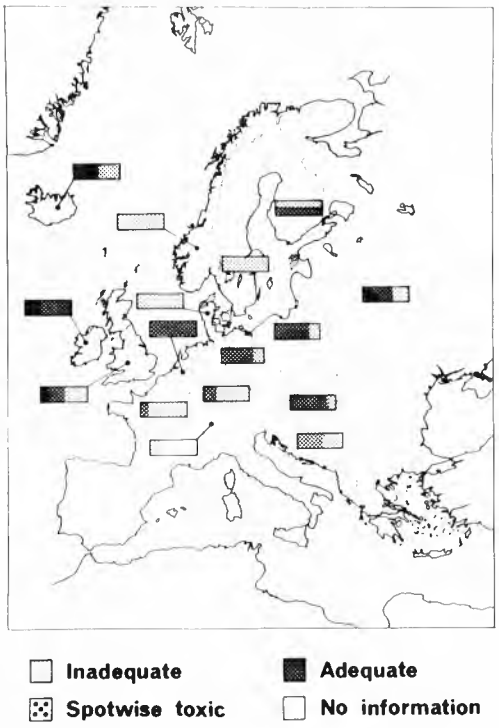

Fig. 1. Distribution of Se in fodder crops in Europe. The two symbols for Finland refer to before and after 1985 (Oksanen & Sandholm 1970; Yläranta, pers. comm.)

Nielsen et al. 1984). From most countries of the world there is no information available about the Se status.

The situation in Europe is illustrated in Fig. 1. There is a geographical pattern in this map showing Scandinavia as a natural low-Se area, while central Europe is balanced between deficiency and sufficiency. From southern Europe information is more infrequent. A few samples from Italy point to a level ranging from somewhat deficient to sufficient (Bordini et al. 1985). Results presented at the International Symposium on Selenium in Belgrade, 1991, indicate that the selenium status in the former Yugoslavia range between adequate and inadequate. Se toxicity is only observed spot-wise in Wales and Eire.

The Se concentrations in plants in areas indicated in Fig. 1 are reflected in the daily Se intake by humans and in their blood Se content. In Table 1 some data are given on this from a few selected countries (Gissel-Nielsen et al. 1984). A great many publications indicate a similar correlation between the Se content of animal food and animal blood Se.

Table 1. Human Dietary Se intakes and Blood Se levels. (Gissel-Nielsen et al. 1984)

	Se intake µg/day	Blood Se µg/l
Belgium	55	123
Canada	98-224	182
China	11-4990	8-3180
Denmark	40	86
Finland before 1985	30	56-87
New Zealand	28-56	59-83
USA	62-216	157-265

Because of this correlation the Se intake of humans and animals living in a certain area can be estimated largely by evaluating the Se uptake by the fodder crops grown in these particular areas.

Se UPTAKE BY PLANTS

A straight correlation between soil Se and plant content exists only when comparing the situation in areas with extreme variations in Se status. Within low to moderate Se areas, no general correlation is present. This is due to the large number of factors influencing the availability of soil Se to plants (Gissel-Nielsen et al. 1984).

In soils with high pH, inorganic Se will be present mainly as selenate, which is hardly fixed at all in the soil. If the precipitation and the leaching is low, most of the Se will be available for the plants, and the crops will be Se-rich. This is known in some places in India. Contrary to this, low pH favours the selenite form, which is fixed strongly in the soil. Even with the same total Se content in the soil as in the above-mentioned example, the Se concentration in the crop might be ten times lower.

This is the situation referred to in Fig. 2, where a fixation of selenite by clay minerals and organic matter is indicated, along with a very strong fixation by iron hydroxides. Through microbial activity volatile Se is lost to the atmosphere. However, Se also returns to the soil from the atmosphere. All this leaves only a minor part of the Se in the cycle to pass through the plant-animal system.

Furthermore, the Se concentrations in plant samples depend upon the time of sampling (Gissel-Nielsen 1975). The release of Se from clay and from organic matter is a very slow process. The Se released during the winter is available for the crops in the spring, when the yield of, e.g., grass is low, giving a relatively high Se concentration. However, as the growth of the grass increases during the summer, less Se is available for the plants, and it is distributed into a much bigger yield, thus the concentration drops as a result of dilution.

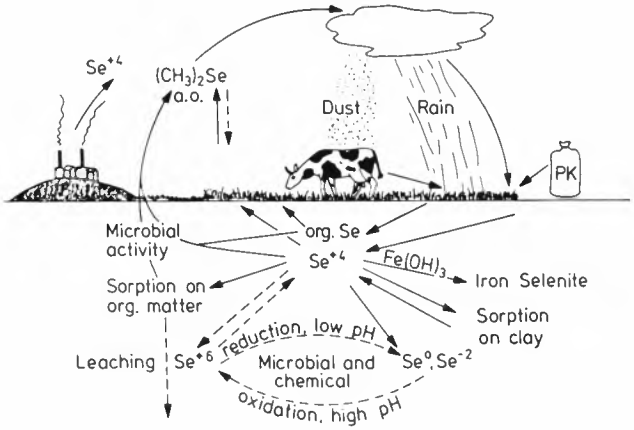

Fig. 2. Possible cycles of Se under field conditions

FIELD TREATMENT WITH Se

To remedy the low natural crop Se concentration, some ways of raising it have been developed over the years, and the most likely means have been studied in a number of field experiments, the main results of which are given in Table 2 (Gissel-Nielsen 1986).

Table 2. Se Treatment of barley in field experiments (Gissel-Nielsen 1986)

Control	0.013 ppm
10 g Se/ha as selenite in PK fertilizer	0.069 -
20 - " -	0.137 -
120 g Se/ha as selenite in PK fertilizer	0.088 -
5 - " - sprayed on plants	0.069 -

These results show that fertilizing with selenate or foliar application of selenium only requires a very small amount of Se to raise the plant content to desirable levels. The selenium can be added in the form of a Se-enriched compound fertilizer or sprayed in mixtures with herbicides or other pesticides.

Another aspect of the field treatment with Se is the chemical form in which the Se is administered to the animals. Whether Se is supplemented through pre-mixed fodder, concentrate, mineral supplement, water, lickstone, or ruminant pellet, the Se is offered as inorganic selenite, while Se in crops is present predominantly as free seleno-amino acids or in proteins. The impact of this application is still subject to much discussion.

BIOAVAILABILITY OF Se

There is, however, no doubt that the bioavailability of different Se compounds varies strongly. This has been demonstrated in several experiments, and in Table 3 the results from just one of them are presented. Laws et al. (1986) fed Se-deficient chickens with a number of Se sources, and in Table 3 the percentage of surviving birds fed the same amount of Se is recorded.

Table 3. Relative biological values of Se (Laws et al. 1986)

Se source	% survival
chick diet	34
+ dried fish	38
+ raw bread	53
+ raw beef	69
+ baked bread	72
+ selenite	81

Table 3 indicates a marked difference in the bioavailability even between organic scources of Se. Such differences stress the importance of the form of Se in animal fodder and human food, and research has been carried out to evaluate the possibilities of influencing the chemical form of the Se in plants. Earlier experiments (Gissel-Nielsen 1987) have shown that Se is translocated in the xylemsap of maize as selenate when added to the nutrient solution as selenate, whilst selenite is metabolized immediately to Se amino acids and translocated as such (Fig. 3).

These results indicate a possible way of affecting the metabolic pathway of Se in plants, so some experiments were carried out in which ryegrass and barley were treated with Se as selenite or selenate through the roots or by foliar application. By using a resin fractionation technique, the water-soluble Se compounds in the grass and the barley grain were separated into Se proteins, Se amino acids, selenite and selenate. This was done with or without previous treatment with pronase. Neither the oxidation state of the added Se nor the method of application had any significant effect on the distribution of the Se between the four possible compounds. In all four cases only about 10% of the Se was present in an inorganic form (Gissel-Nielsen 1987). This implies that all means of supplementation of crops with inorganic Se have the same impact on the distribution of Se between different compounds. Thus, their value as Se supplements can be judged on the basis of the uptake in percent of the total added.

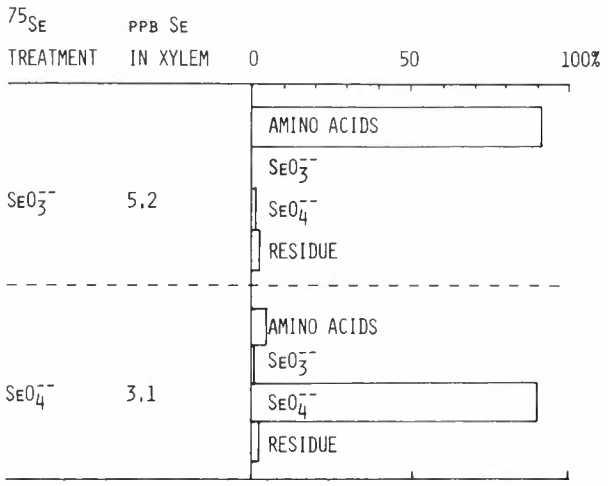

Fig. 3. The distribution of Se into fractions of xylem sap from maize when added as selenate or selenite

CONCLUSION

The human and animal intake of Se from natural sources varies throughout the world from a few cases of toxicity to rather widespread incidences of moderate to strong deficiency. Only China has reported severe cases of deficiency leading to the death of many humans, but the moderate deficiency, as observed in many countries, might be more dangerous to human health than is often realized. In 1985 Finland considered the consequences of high rates of cardiovascular diseases and certain forms of cancer and by law introduced Se in all compound fertilizers in amounts that equal 3-8 g Se/ha as selenate. In other countries, such as e.g. New Zealand selenized compound fertilizers are available. In many countries

Se-enriched, pre-mixed fodder for the livestock and Se pellets for human supplementation are available, but most often the Se is present in an inorganic form. This means that in many cases the Se nutrition of animals is taken much better care of than that of people, but, in general, people have a much more mixed diet that includes more of the relatively Se-rich foodstuffs such as fish and some vegetables.

In the future increased attention will be paid to the importance of a sufficient Se content in human food - as well as in that of livestock. As shown in this paper, a reasonable uptake of Se by our crops is a cheap, sure, and easy way of ensuring a desirable human and animal intake of Se.

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14 Selenium fertilization in Finland: selenium soil interactions

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The natural selenium (Se) content in Finnish agricultural plants is very low. The average selenium content of cereal and grass crops, for example, has been 0.01-0.02 mg/kg of dry matter. The reason for the low concentration in Finnish plants is not the small selenium content in the soil, which in Finnish agricultural soils usually varies between 0.1 mg/kg and 0.6 mg/kg. Water-extractable selenium provides a better picture of the soil selenium availability to plants than total selenium content. On average, only 4% of the total selenium present in the soil is extractable in hot water. In Finland the addition of selenium in the form of selenate to the usual multinutrient fertilizers used in agriculture and horticulture was introduced on 1 July 1984. Initially, the selenium added to the fertilizers used mainly for cereal crops was at the rate of 16 mg/kg, and that to the fertilizers used mainly for grassland crops at 6 mg/kg. Thus, the amount of selenium introduced with the fertilizer per crop was about 8 g/ha for cereals and 3 g/ha for grasses. The selenium content target for plants was set at 0.1 mg/kg of dry matter, which clearly was often exceeded. Therefore, in 1991 the rate of selenium addition to the fertilizers was decreased to 6 mg/kg of fertilizer. No statistically significant increase in plant available selenium content in soils has been detected.

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SELENIUM IN AGRICULTURAL SOILS

The selenium (Se) content in Finnish agricultural soil usually varies between 0.1 mg/kg and 0.6 mg/kg (Sippola 1979; Yläranta 1983a). The highest selenium content, some cases exceeding 1 mg/kg, is found in organogenic soils (Table 1). The selenium content in mineral soils is closely dependent on the clay fraction of the soil (Yläranta 1983a).

Since the selenium content in agricultural soils varies in different parts of the world, usually between 0.1 and 0.2 mg/kg (Swaine 1955, p. 91), that in Finnish agricultural soil cannot be described as exceptionally low.

Water-extractable selenium provides a better picture of the soil's selenium availability to plants than total selenium content. On average, only 4% of the total selenium present in Finnish soil is extractable in hot water.

Table 1. Soil sample means ($\bar{x}$), standard deviations (s) and ranges for total selenium content (Yläranta 1983a)

Soil group	No. of samples	Total Se (mg/kg) $\bar{x}$	s	Range
Plough layer (0-20 cm)				
1. Mineral soils	93	0.209	0.108	0.050-0.633
Clay soils	29	0.290	0.120	0.131-0.633
Coarse mineral soils	64	0.172	0.078	0.050-0.489
2. Organogenic soils	19	0.464	0.239	0.212-1.281
Deeper layer (20-40 cm)				
1. Mineral soils	80	0.197	0.115	0.040-0.654
Clay soils	26	0.275	0.125	0.140-0.654
Coarse mineral soils	54	0.159	0.089	0.040-0.560
2. Organogenic soils	13	0.575	0.178	0.329-0.982

The chemical form of inorganic selenium in the soils depends largely on the pH and oxidation-reduction potential (Eh) of the soil. Three oxidation states of selenium are stable within the Eh range existing in the environment, +6 (selenate), +4 (selenite), and 0 (elemental selenium).

The predominant mobile inorganic forms of selenium are selenate in aerated alkaline soils, and selenite in acid, reducing soils such as those that generally occur in humid areas (Geering et al. 1968).

The selenium-sorbing mineral constituents of soils are Al and Fe oxides, clay minerals, and calcite (Goldberg & Glaubig 1988). Selenite binds strongly with the active iron oxides in the soil, with the clay fraction and with the organic matter (Gissel-Nielsen 1976; Hamdy & Gissel-Nielsen 1976; John et al. 1976; Hamdy & Gissel-Nielsen 1977). In Finland, the selenium content of mineral soils is closely dependent on the clay fraction of the soil (Yläranta 1983a).

Ions with low affinity for a proton also have a low affinity for the metal ions forming part of the surface oxides in the soil (Barrow & Bowden 1987); hence, it is predicted that the binding constant for selenate ions in soil will be low (Barrow & Whelan 1989).

RAISING THE SELENIUM CONTENT IN PLANTS

The natural selenium content of Finnish agricultural plants is very low. At least the selenium content in young wheat plants is lower than that of any other country from the 30 countries around the world included into the study published by Sillanpää & Jansson (1992).

The selenium content in cultivated crops can be raised most effectively by adding selenites or selenates to fertilizers, spraying these salts onto the crops or by treating the seeds with aqueous solutions of selenium compounds. The most suitable selenium compounds for this purpose are selenites and selenates (Bisbjerg & Gissel-Nielsen 1969; Gissel-Nielsen & Bisbjerg 1970; Gissel-Nielsen 1977; Yläranta 1985).

The addition of selenate to the most common multinutrient fertlizers used in agriculture and horticulture was introduced in Finland in 1984. Initially, selenium was added

at the rate of 16 mg/kg to the fertilizers used mainly for cereal crops, and to those used mainly for grassland cultivation at the rate of 6 mg/kg. As the average fertilizer application is about 500 kg/ha, each cereal crop received 8 g/ha of selenium and each grass crop 3 g/ha.

Addition of Se to fertilizers raised the Finns' selenium intake from about 0.03 mg/d in the mid-1970s to 0.1-0.12 mg/d in the late 1980s.

The selenium content target for plants was set at 0.1 mg/kg dry matter (Yläranta 1985), but clearly the target was often exceeded. Therefore, in 1991, the rate of Se added to the fertilizers was decreased to 6 mg/kg of fertilizer.

Selenium from selenate finds its way into plants more efficiently than that from selenite, irrespective of whether the selenium is applied to the soil or directly onto the plants. The reason is the high solubility of selenate.

Applications of selenate fertilizer and seed treatment, e.g. in cereal crops with selenate, are probably about equally effective. Treating seeds reliably and safely with concentrated and toxic solutions of selenium salts is not easy, and this method cannot therefore be recommended for general practice. It is impossible to compare the effectiveness of selenium fertilizer with that of foliar spraying in cereal crops and plants that cover only part of the ground. In the case of cereals, for example, foliar spraying of selenite is more effective the greater the ground coverage by the plants. The effectiveness of foliar spraying is also influenced by the spraying conditions and even by the soil type at the site, as selenium from that part of the spray falling onto the ground, particularly the readily soluble selenate, reaches the plants via the roots (Yläranta 1985).

In addition to selenate fertilizer, spraying with selenate is also suitable for use in practice. The effect on the selenium content of the grain crops of spraying with selenite depends greatly on the stage of growth of the plants at the time of spraying. Selenate is thus more suitable than selenite for spraying, as it is not always possible to choose the spraying time exactly and consistently. Selenite and selenate sprayings are almost equally effective in raising the selenium content in grass, e.g. timothy grass.

Most of the selenium added to the acid agricultural soils of Finland in the form of selenite fixes quite rapidly into a form that is poorly available to plants. Sorption of selenite by soil seems to be weaker, however, than that of phosphate (Yläranta 1983b).

Selenium added to soil in the form of selenate remains in a readily soluble form for several months. On most Finnish agricultural soils selenium lost through volatilization or leaching is probably small. The low selenium content the plants in the year following the selenium treatments thus confirms the assumption that selenium applied to Finnish agricultural land as selenite and selenate is susceptible to reduction and fixing. On coarse mineral soils and soils with a high humus content, however, treatment with selenate had a marked residual effect on the selenium content of barley. This indicates that some of the selenium from the selenate remains available to the plants for at least two growing seasons (Yläranta 1985).

FACTORS AFFECTING THE UPPTAKE BY PLANTS OF SELENIUM ADDED TO SOIL

The Finnish agricultural soils are mainly acidic in nature, so liming can be assumed to increase the uptake of selenium by plants. In pot experiments in which liming with $Ca(OH_2)$ was used to raise the pH ($CaCl_2$) of clay, fine sand and Carex peat from 4.4-4.6 to 6.1-6.3, liming raised the selenium content of rye grass only in the treatment of Carex peat (Yläranta 1983c).

Although the use of fertilizers containing phosphate and sulphate should not be expected to bring about major changes in the solubility of soil selenium (Cary & Gissel-Nielsen 1973), they may cause some interactions in the soil.

It is fairly evident that selenate is anlogous with sulphate. The appropriate analogy for selenite is phosphate (Barrow & Whelan 1989). It has been found that sulphur, in the form of sulphate, effectively reduces the uptake of selenium by plants from selenate, but reduces the uptake only slightly from selenite (e.g. Gissel-Nielsen 1973). Correspondingly, selenate can be assumed to interfere with the sulphur economy of plants (Milchunas et al. 1983). This has raised further doubts about the suitability of applying selenate to soil as a source of selenium for plants.

Therefore, in a new pot experiment in peat, clay and sandy loam soil, an effort was made to get a clearer picture of the effects of liming, sulphate and phosphate on the selenium content of plants (Yläranta 1990). It was hoped that new information would be obtained by using much more powerful liming and sulphate and phosphate additions than those used in practical cultivation measures.

Two-litre polythene pots were used in the experiment. There were four replicates, the plan being as follows (Yläranta 1990):

Control, no addition of selenium
Selenite addition, Se 0.200 mg/pot
Selenate addition, Se 0.200 mg/pot
Selenite + liming
Selenate + liming
Selenite + phosphate, P 800 mg/pot
Selenate + phosphate, P 800 mg/pot
Selenite + sulphate, S 800 mg/pot
Selenate + sulphate, S 800 mg/pot

The pH ($CaCl_2$) of the unlimed clay and sandy loam soils at the end of the experiment averaged 4.7 and 4.8, and that of limed soils 6.4 and 6.5. The corresponding pH values for peat soil were 3.9 and 5.8 (Table 2). The addition of sulphate clearly increased the content of soluble salts in all soils.

The addition of sulphate decreased the selenium concentrations in selenate treatment on all soils in both cuts (Table 3, first cut). The addition of phosphate decreased the Se concentrations in the selenate treatment only in the second cut on clay and sandy loam.

In selenite treatments, liming increased the selenium concentration in all soils in both cuts, but the increase was statistically significant only in a clay and peat soil in the second

cut. In the selenate treatment, liming decreased the selenium concentration in the first cut on all soils and in the second cut on sandy loam soil.

Table 2. Mean pH ($CaCl_2$) of soils in different treatments after the second cut (Yläranta 1990)

Treatments	Clay	Sandy loam	Carex peat
Control, no Se addition	4.7	4.7	3.9
Selenite addition	4.8	4.7	3.9
Selenate addition	4.7	4.7	3.9
Selenite + liming	6.4	6.5	5.8
Selenate + liming	6.4	6.5	5.8
Selenite + phosphate	4.8	4.7	3.9
Selenate + phosphate	4.8	4.7	3.9
Selenite + sulphate	4.8	4.7	3.9
Selenate + sulphate	4.7	4.7	3.9

Table 3. Mean selenium content of rye grass at first cut, mg/kg dry matter (Yläranta 1990). Figures given for each soil applied with selenium and not marked with a common letter differ from each other at the 5 % level of significance (Duncan 1955). The control treatment is not included in the statistical comparison

Treatments	Clay	Sandy loam	Carex peat
Control, no selenium added	0.009	0.010	0.009
Selenite addition	0.159^{dc}	0.359^{d}	0.523^{cd}
Selenate addition	2.19^{a}	2.56^{a}	2.90^{a}
Selenite + liming	0.278^{cd}	0.396^{d}	0.725^{c}
Selenate + liming	1.89^{b}	2.03^{b}	1.94^{b}
Selenite + phosphate	0.144^{de}	0.334^{d}	0.238^{d}
Selenate + phosphate	2.10^{a}	2.55^{a}	2.63^{a}
Selenite + sulphate	0.120^{e}	0.335^{d}	0.438^{cd}
Selenate + sulphate	0.391^{c}	1.11c	0.680^{c}

SELENIUM FERTILIZATION, SOIL SELENIUM AND THE ENVIRONMENT

According to the results of field experiments, cereal crops above the ground accumulate 5-20% of the selenium applied in the form of selenate fertilizer (Yläranta 1985). The selenium uptake of grasses is about 20%. The selenium fertilization method practised in Finland indicates that apparently the selenium intake of plants is clearly higher than the experimental figures (e.g. Yläranta 1990). However, if only a small proportion of selenium applied to the soil is removed each year along with crop, the selenium content in the soil will rise.

The annual selenium application is now about 3 g/ha, for instance for cereal crops and clearly less than 10 g/ha for grasses, although farmers can apply selenized fertilizers for two to three cuts yearly. It will take many years before it is possible to analyse the increase

of the total content of soil selenium. Therefore, it is more useful to control the plant-available selenium fraction in the soil.

From 1982 to 1984 farmers ordered about 700-800 selenium analyses annually from the Soil Fertility Service of Finland (Table 4). Since the addition of selenium to fertilizers, the annual number of selenium analyses ordered has clearly decreased (Yläranta 1990).

Table 4. Soil sample means and range (1990) for hot water extractable selenium content (Se mg/l of soil, plough layer) in Finland in 1982-86 and 1990

Soil group	Sampling year	No. of samples	Se mg/l of soil	
			Mean	Range
Coarse mineral soils	1982	646	0.007	-
	1983	514	0.007	-
	1984	603	0.007	-
	1985	286	0.009	-
	1986	155	0.008	-
	1990	290	0.006	0.002-0.041
Clay soils	1982	71	0.007	-
	1983	78	0.008	-
	1984	86	0.011	-
	1985	27	0.009	-
	1986	14	0.009	-
	1990	98	0.006	0.003-0.017
Organogenic soils	1982	94	0.007	-
	1983	102	0.006	-
	1984	128	0.010	-
	1985	69	0.008	-
	1986	38	0.007	-
	1990	62	0.006	0.002-0.010

The selenized fertilizers were in use throughout the farming area for the first time during 1985, although it was possible to use some selenium fertilizers as early as in 1984. Thus, the selenium-enriched soil samples have been avaiable since 1984. Those selenium concentrations given in Table 4 are between 1982 and 1986 from samples ordered by farmers from the Soil Fertility Service of Finland. Only mean values are presented. In 1990 samples for selenium determinations were collected in such a way that they covered different cultivated soils and sample sites around Finland. It is impossible to find any statistically significant increase in hot water extractable selenium content caused by selenium fertilization.

In searching for suitable ways of raising the selenium content of plants in actual production, the possible effects of selenium on the environment must also be taken into account. Selenium, especially in the form of selenate, has caused environmental problems in some regions of the world (e.g. Ohlendorf 1989). This kind of situation occurs primarily under arid or semi-arid conditions, but not so readily in humid regions, such as in Finland.

It is still very important to know in greater detail the reduction and fixation speed of added selenate under different conditions.

In order to evaluate the effects of selenium application on the environment, it is necessary to know the susceptibility of selenite and selenate to leaching from the soils and the factors affecting this. A leaching experiment conducted by Yläranta (1982) showed that selenate can be leached at least from peat soils. Yläranta (1991) measured greater selenium leaching for clay, sandy loam and Carex peat soil (Table 5) than that found in the earlier experiment (Yläranta 1982).

Table 5. Leaching of selenite and selenate added to the soil from a 20-cm soil column, expressed as a percentage of the selenium added (Yläranta 1991). Figures given for each soil not marked with a common letter differ from each other at the 1% level of significance (Duncan 1955). The mean pH ($CaCl_2$) values at the end of the experiment were 4.7, 4.6 and 3.9 for clay, sandy loam and Carex peat, respectively

Treatment	Clay	Sandy loam	Carex peat
Selenite addition	0.0^{c}	1.4^{c}	2.0^{c}
Selenate addition	11.9^{c}	70.9^{b}	75.4^{b}
Selenite + phosphate	0.5^{c}	3.9^{c}	1.6^{c}
Selenate + phosphate	37.2^{b}	79.1^{ab}	68.9^{b}
Selenite + sulphate	2.7^{c}	4.8^{c}	5.7^{c}
Selenate + sulphate	65.8^{a}	91.2^{a}	93.0^{a}

However, it appears to be difficult to find a sound scientific basis for the fear that selenium fertilization would pose a serious environmental risk in Finland. It is wrong to compare the Finnish acid and reducing soil conditions to conditions where selenium can remain or even oxidize to the valence state six, which is a very soluble form of selenium. This kind of situation occurs primarily under arid or semi-arid conditions.

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15 Selenium fertilization in Finland: selenium content in feed and foods

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Varo, P. 1993. Selenium fertilization in Finland: selenium content in feed and foods. Norwegian Journal of Agricultural Sciences. Supplement No. 11: 151-158. ISSN 0802-1600.

It was already known in the 1960s that the feedstuffs produced in Finland contained very small amounts of selenium (Se). The average daily Se intake in the mid-1970s was 25 μg/d/person. In 1984 the Ministry of Agriculture and Forestry made a decision that multielement agricultural fertilizers should from then on be supplemented with sodium selenate. The original levels of supplementation were 6 mg Se/kg fertilizer to be used in the production of grass and hay, and 16 mg Se/kg for the grain production. The supplementation practice was adjusted at the beginning of 1991 to only one level of added Se, 6 mg/kg fertilizer. The Se supplementation practice of fertilizers affected the average intake conspicuously. For the average Se intake, a plateau of 110-120 μg/d was reached in 1987, and it stayed constant until 1991. The reduction in the amount of Se in fertilizers has caused a drop, and in 1992 the estimated intake was about 90 μg/d. This is close to the present understanding of the adequacy of Se in diets.

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It was already known in the 1960s that the feedstuffs produced in Finland contained very small amounts of selenium (Se) (Oksanen & Sandholm 1970). This was connected with the high prevalence of severe animal diseases such as muscular dystrophy or exudative diathesis, for example. In the 1970s it was also shown that all Finnish agricultural products contained exceptionally low amounts of Se (Koivistoinen 1980). The average Se intake of the Finnish population varied somewhat according to the extent of grain imports, but in general it was as low as 25 μg/10 MJ (2400 kcal).

In 1984 the Ministry of Agriculture and Forestry made a decision that multielement agricultural fertilizers should from then on be supplemented with sodium selenate. The original levels of supplementation were 6 mg Se/kg fertilizer to be used in the production of grass and hay, and 16 mg Se/kg for the grain production. The supplementation practice was adjusted at the beginning of 1991 to only one level of added Se, 6 mg/kg fertilizer.

THE EFFECT OF Se SUPPLEMENTATION ON FEEDSTUFFS: GRASS AND HAY

Before 1985 the Se content of grass and hay was around 10 μg/kg dry matter. It can be

seen from Fig. 1 that the Se supplementation of fertilizers has had a considerable, but not very even effect on the Se concentration of silage and dry hay. The annual averages have increased to about 150 μg/kg dry matter (DM). Individual farms have produced silage containing more than 1 mg Se per kg dry feedstuff. The reason for such high values is not clear, since, according to the farmers, the normal fertilization and silage preparation practices have been followed. Most of the peak values will probably be cut off with the lowering of the amount of supplemented Se in fertilizers.

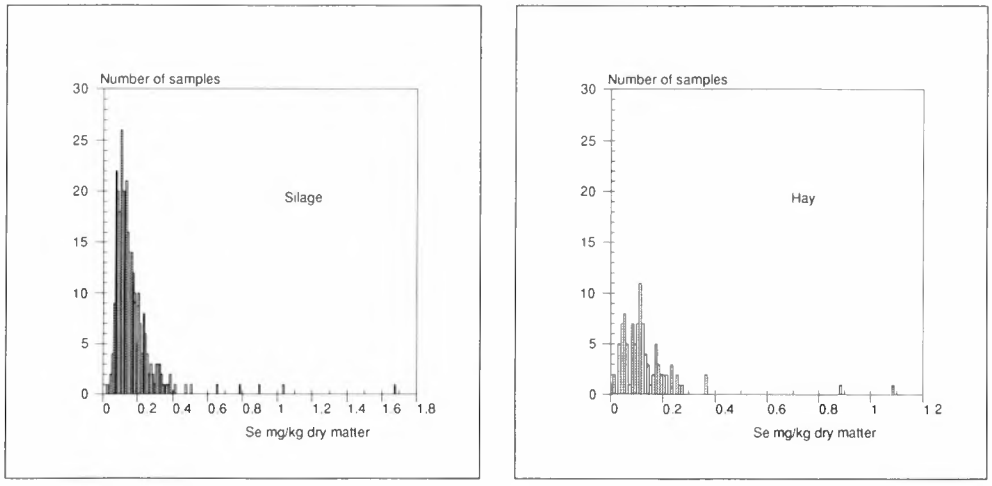

Fig. 1. The Se concentration of silage and hay from different farms in 1989

GRAIN

The primary target of the Se supplementation of fertilizers is cereal grains, although the treatment is known to affect all other agricultural products, as well. The original Se concentration of all cereals grown in Finland was 10 μg/kg or below (Koivistoinen 1980). In comparison, the Se concentration of European cereals varies usually between 20 and 50 μg/kg, and that of North American cereals between 200 and 500 μg/kg (Varo & Koivistoinen 1981).

Figure 2 shows the Se content of Finnish grown cereal grains before and after starting the Se supplementation practice. In the case of spring wheat, the average Se content was between 250-300 μg/kg during the period 1986-90 (Eurola et al. 1990). In 1991, after decreasing the Se level of fertilizers, it sank to its present level, about 125 μg/kg.

In winter cereals, rye and winter wheat, the annual averages have varied between 20 and 70 μg/kg. The main reason for this low response most likely is the fertilization practice, which is different from that of spring sown cereals. In the autumn, in connection with sowing, both rye and winter wheat are usually supplied with a small amount of Se supplemented fertilizer. The main fertilization is given in May, after the initiation of the actual growing season, but now it is more usual to spread plain, non-supplemented N-

fertilizer. However, leaching of selenate, as well as reduction from selenate to selenite and the consequent binding to the soil during the winter season may also be effective.

Oats and barley are cereals grown mainly for animal feeding purposes. They are spring sown cereals, and their response to the Se supplementation has been almost identical to that of spring wheat. Their annual averages have varied from 220 to 260 μg Se/kg.

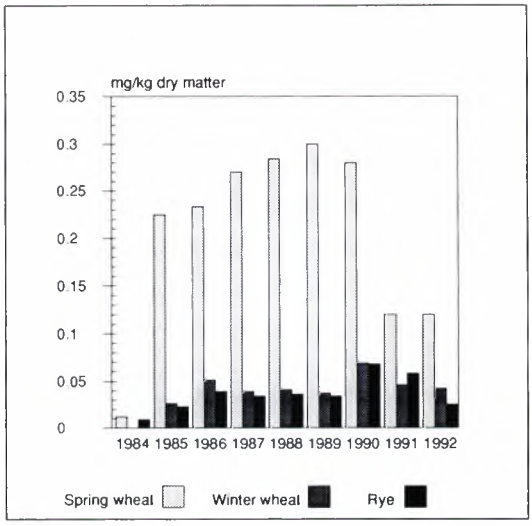

Fig. 2. The selenium content of Finnish grown grain in 1984-92

Figure 3 shows the farm-to-farm variation in the content of Se in barley produced in 1990 and 1992. The great majority of the concentrations lay between 100 and 300 μg/kg in the harvest of 1990. However, some of the farms produced grain of almost original Se content (about 10 μg/kg), while the grain of some other farms exceeded the average level several-fold. These minimum and maximum values may have an important bearing on animal feeding, since, throughout their lives, the farm animals eat fodder produced on one single farm. The use of a lowered level of supplemented Se has effectively cut out the high Se grains, as can be seen in the 1992 graph.

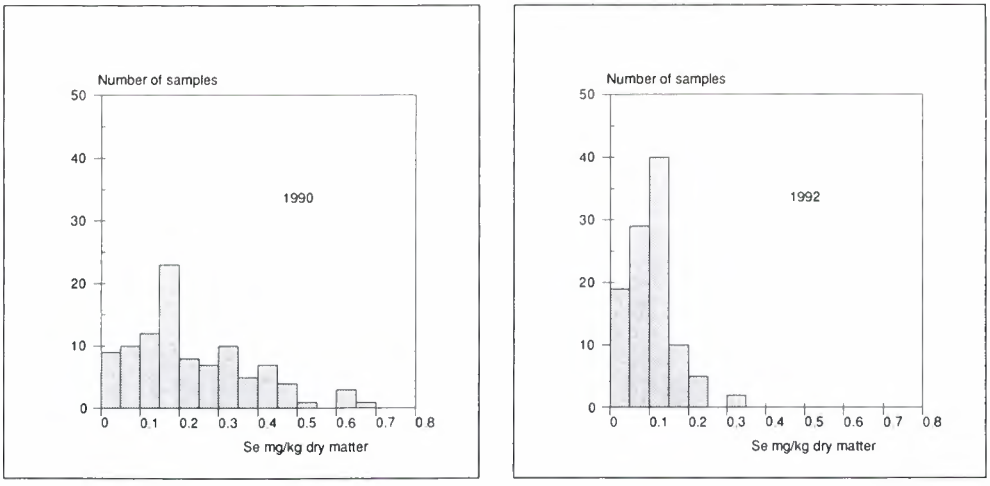

Fig. 3. The distribution of selenium concentrations in barley from differemt farms in 1990 and 1992

Finland sometimes has to import grain because of an inadequate or low quality harvest. Then, domestic and imported grains are mixed for milling purposes in ratios issued in the statutes. In recent years, a significant part of the imported grain has been of North American origin. It has, for some periods, raised the Se content of flour and bread, even during the years of Se fertilization.

Grain imports are also the reason for the year-to-year fluctuation in the average Se intake, which was quite significant prior to the Se fertilization practice. In the years of full self-sufficiency in grain production, the minimum Se intake varied between 20 and 30 μg/d. Imported grain could increase the intake up to 50 μg/d or higher (Koivistoinen & Varo 1987).

During the years 1985-90, cereal products have contributed about 20% (25 μg/d) of the total daily Se intake. The cereal consumption in Finland is about 200 g/d (120 g wheat, of which approximately 15% is winter wheat, 50 g rye, 5 g barley, 10 g oats, and 15 of rice and other imported cereals) (National Board of Agriculture 1991). Since 1991, the Se content of cereals has decreased, and their contribution to the Se intake has been reduced to about 15 μg/d at the present level of supplementation.

MEAT PRODUCTS

In the mid-1970s the Se content of beef produced in Finland was especially low, the average concentration being about 50 μg/kg DM. Pork contained more Se (about 200 μg/kg DM), probably because pig growers may have fed their pigs with selenite-supplemented commercial feedstuffs quite regularly. Even this level was low compared to reported values from other countries (Koivistoinen 1980).

Selenite is less effective than organic Se in raising the Se content in musculous tissues (Ekholm et al. 1991). This explains the dramatic effect of the Se fertilization on the meat Se. The concentration in beef in 1990 was about 600 μg/kg DM (an over ten-fold increase from the mid-1970s), and that in pork about 1100 μg/kg DM (Fig. 4). The Se content in broiler chicken was about 800 μg/kg DM in 1991.

The Se content in liver is about twice as high as that in meat: there was about 1400 μg Se in a kilo of bovine liver (DM), and about 2400 μg/kg in porcine liver (DM) in 1990. Other tissues respond generally less than meat and liver to organic Se (Ekholm et al. 1990).

During the Se fertilization practice, meat and meat products have become the most significant category of foods as a source of Se. The contribution to the total Se intake is about 40% (nearly 50 μg/d). After decreasing the level of fertilizer Se, first signs of the drop in meat Se were observed in December 1991, and new plateaux have evidently been reached by now. The relative significance of meat products as a source of Se has remained very high; in 1992 it was 46%.

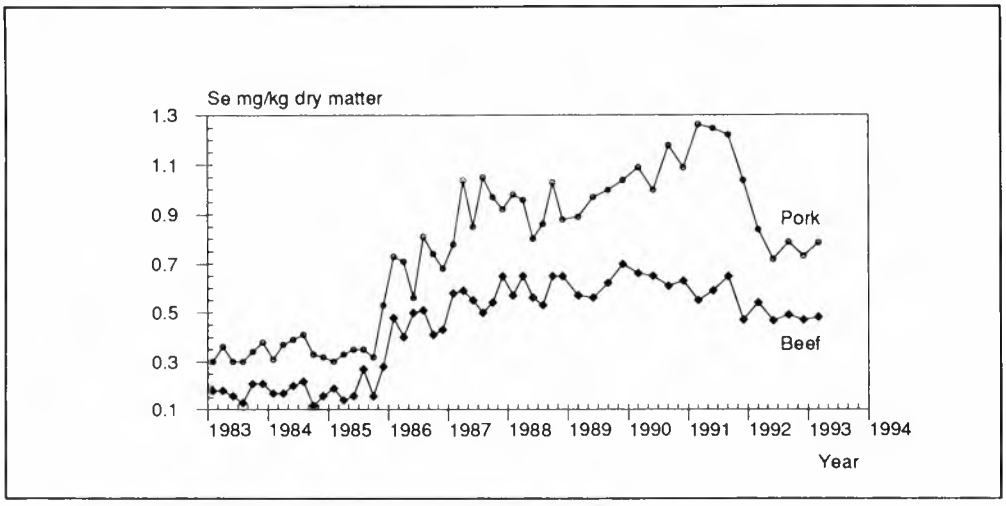

Fig. 4. Changes in the Se content of pork and beef

DAIRY PRODUCTS AND EGGS

In the mid-1970s the Se concentration in milk was very low, about 30 μg/kg DM (3-4 μg/l). In the early 1980s, it slowly increased, perhaps because of the increasingly intensive use of selenite supplemented commercial feeds, mineral concentrates, and Se medication.

The effect of Se supplemented fertilizers on milk composition was rapid and substantial. The practice of Se supplementation was started in May 1985. Already the milk samples collected in June 1985 contained about 130 μg Se/kg DM compared with the earlier level of 50 μg/kg DM. The plateau, 175 (summer)-225 (winter) μg/kg DM was reached in a few months (Ekholm et al. 1991a). In 1991, after decreasing the amount of Se in fertilizers, milk was again the fastest to respond: a distinct downward trend in milk Se was observed in the summer of 1991.

The Se level in cheese is nearly double that in milk. This is due to the high protein content of cheese. At its highest the concentration was about 430 μg/kg DM. The present Se concentration in cheese is between 250 and 300 μg/kg DM.

The Se content in eggs was relatively high (about 450 μg/kg DM) by the mid-1970s because of the use of Se-rich feedstuffs. After 1985 the concentration gradually rose to the level of 1200 μg/kg DM, but the present level is 800-900 μg/kg.

The contribution of dairy products and eggs to the total Se intake was about 30 μg/d (over 25%) in 1990, while in 1992 it was 20 μg/d (23%).

OTHER FOODS

Fish

In the years before Se supplementation the relative importance of fish as a source of Se was much higher than it is today. The earlier contribution varied from 10 to 25% of the total, alternating with the degree of grain imports. The present relative significance is about 6%.

The Se content in fish has remained unchanged. It usually lies between 500 and 1500 μg/kg DM, depending on the species and the fat content in fish (Eurola et al. 1991). Consequently, the average amount of Se received from fish is about 5 μg/d/person.

Potato

The Se fertilization has increased the Se content in potatoes at least tenfold compared to the level of the mid-1970s. The level in 1990 was around 100 μg/kg DM, which means that an average 3 μg/d was received from potato. Potato grown in 1992 contained about 50 μg Se/kg.

Vegetables

It has been found that the response to the supplementation practice varies according to species (Eurola et al. 1989). Tomato, for instance, is a low responder (present concentration about 30 μg Se/kg DM), while high concentrations are encountered especially in different crucifers, like white cabbage (600 μg Se/kg DM) or cauliflower (700 μg/kg DM).

Fruit and berries

Most of the fruit consumed in Finland is imported. Of cultivated fruit and berries, only apple, strawberries and currants are produced locally in significant quantities. Even their Se content has remained low: apple 10 μg/kg DM, blackcurrant 40 μg/kg DM. The Se content in wild berries, like lingonberry and bilberry, is on the original low level, way below 10 μg/kg DM.

The contribution of vegetables, fruit and berries to the total Se intake is almost insignificant. Only about 1 μg/d is received from this category of foods, on average.

Mothers' milk

The Se level in the breast milk of Finnish mothers in the mid-1970s was 50 μg/kg DM. Since the beginning of the supplementation practice, the level has stayed constantly between 105 and 125 μg/kg DM. This concentration is only one-half that of the average cow milk level, reflecting mainly the difference in the protein content of these two types of milk.

SELENIUM INTAKE

The average daily Se intake in the mid-1970s was 25 μg/d/person. At that time the grain imports were insignificant for a long period, at least from 1972 to 1978. In some years in the early 1980s, the intake was 40-50 μg/d, mainly as a result of grain imports (Varo et al. 1988). However, the Se intake was almost uninterruptedly below 50 μg/d, which was the lower limit of the safe and adequate intake for Se defined by the US National Academy of Sciences (1980).

The initiation of the Se supplementation practice of fertilizers affected the average intake conspicuously (Varo et al. 1993). The effect of Se fertilization on some foodstuffs can be seen in Table 1. For the average Se intake, a plateau of 110-120 μg/d was reached in 1987, and it stayed constant until 1991. The reduction in the amount of Se in fertilizers has caused a drop, and in 1992 the estimated intake was about 90 μg/d. This is close to the present understanding of the adequacy of Se in diets (US National Academy of Sciences 1989; Nordisk Ministerråd 1989).

Table 1. Trends in Se content of Finnish retail-store foodstuffs[a)]

	1984		1990		1992	
	n	mg/kg dry matter	n	mg/kg dry matter	n	mg/kg dry matter
Wheat bread, French loaf	24	0.05 ± 0.04	16	0.23 ± 0.02	16	0.16 ± 0.03
Rye bread, whole	24	0.07 ± 0.05	16	0.06 ± 0.02	16	0.06 ± 0.03
Potato	2	<0.01	16	0.11 ± 0.03	16	0.06 ± 0.02
Beef, steak	24	0.17 ± 0.06	16	0.64 ± 0.08	16	0.49 ± 0.05
Pork, fillet	24	0.35 ± 0.07	16	1.09 ± 0.09	16	0.77 ± 0.09
Milk, whole	24	0.06 ± 0.01	16	0.23 ± 0.02	16	0.15 ± 0.02
Cheese, Edam type	24	0.09 ± 0.02	16	0.42 ± 0.04	16	0.29 ± 0.02
Egg	24	0.69 ± 0.15	16	1.27 ± 0.13	16	1.00 ± 0.18

[a)] Application of Se supplemented fertilizers was started in 1985, the amount of Se in fertilizers was reduced in 1991

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16 Selenium fertilization in Finland: effect on milk and beef production

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The selenium required by animals is obtained in organic or inorganic form and its nutritional availability varies with the compounds in which it occurs. The availability is greater in organic compounds than in inorganic form. In a series of trials it was found that selenium-enriched silage increases the selenium content of milk and beef more than the sodium selenite at the same selenium level in the diet. The selenium level in basic feeds, 0.15 to 0.20 mg Se/kg DM, seems to be sufficient in milk and beef production and gives a selenium content for milk of 0.015-0.020 mg/l and that for beef of 0.15-0.20 mg/kg fresh weight. These concentrations are obtained by the addition of selenium to fertilizers at a level of 6 mg/kg, which is that used in Finland.

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The assimilation of selenium by the animal and its different tissues depends on many factors. These include the structure of the selenium compound, and the amount of selenium already accumulated in the system. In addition, the various factors connected with selenium metabolism each exert their own influence; among these are the content of vitamin E and unsaturated fatty acids and the amounts of sulphur, arsenic, cadmium and mercury in both the feeds and the animal tissues (cf. e.g. Underwood 1971; Vokal-Borek 1979; Oksanen 1980). The mechanism involved is thus very complex, so that the question of selenium should not be dealt with in isolation, but as part of a larger whole. This is also the case in studies of the selenium content of domestic animal products and the factors on which it depends.

In ruminants, the rumen microbes are able to metabolize inorganic selenium and to bind it to their microbial protein in the same way as organic selenium, in the form of selenoamino acids (Whanger et al. 1967; Hidiroglou et al. 1968, 1974). Part of the selenium is reduced in the rumen to insoluble form and, being unavailable to the animal, is eliminated with the faeces (Cousins & Cairney 1961; Butler & Peterson 1961; Jakobsson 1966a). The anaerobic strongly reducing conditions in the rumen may contribute to reduce the selenium compounds.

The selenium content of the muscles is fairly low. The selenium content of the carcass is fairly evenly divided between the muscles and the bones, while the fatty tissues contain very little selenium (Lopez et al. 1968; Ekholm et al. 1991).

Selenium leaves the animal body in faeces and urine, through the respiration and by secretion into milk. In non ruminants it is chiefly excreted in urine, but in ruminants most of the selenium is excreted in the faeces (e.g. Cousins & Cairney 1961; Peterson & Spedding 1963; Aspila 1991).

The greater part of the selenium occurring in milk is bound to the proteins. Thus, the secretion of selenium into milk is connected with the synthesis, transport and secretion of proteins in the mammary epithelial cells (Allen & Miller 1981). The proteins undergo many structural changes during their migration and secretion, and it is still uncertain at what stage of this process selenium is bound to the milk protein.

The selenium content in milk depends on the selenium content in the feed ration and also on the form in which selenium occurs. Organic selenium is more available for secretion into milk than is inorganic selenium (Jakobsson et al. 1965; Conrad & Moxon 1979; Aspila 1991). This is evidently due to the fact that, being an amino acid, selenomethionine is able to bind directly to lactoprotein molecules (Jakobsson et al. 1965).

SELENIUM EXPERIMENT WITH DAIRY COWS AND GROWING CATTLE

In 1981 an extensive research project was commenced at the Department of Animal Husbandry of the University of Helsinki. Its purpose was to examine the possibility of increasing the selenium content in milk, beef and other foodstuffs of animal origin by adjusting the selenium supplementation in animal feeds. In this way the supply of selenium could be raised in the diet of the Finnish people.

Materials and methods

Milk and meat production trials were begun with the preparation of selenium-enriched silages (grass was Se-sprayed), attention being paid to the assimilation of selenium by grass and losses during preservation (Aspila 1991). The research also included digestibility and balance trials and physiological studies on the suitability of various selenium feeds as sources of selenium for ruminants. The results of these experiments are to be presented elsewhere.

The purpose in the milk production experiment was to study the most appropriate source and supplementation level of selenium to raise selenium content in milk as well as the effect of selenium supplementation on the production and health of the animals. The experiment was of a 2 x 2 factorial design, with sodium selenite and selenized silage as main effects (during period V selenized barley, Table 1). There were thus four experimental groups:

Group 0: No sodium selenite
Group 1: Sodium selenite } Control silage

Group 2: No sodium selenite
Group 3: Sodium selenite } Selenized silage

The experiment involved a total of 48 dairy cows and comprised six periods with different levels of selenium intake (Table 1). The details of the experiment and feeding of the animals are presented elsewhere (Aspila 1991).

Table 1. Experimental periods and selenium content of the diet

Period	Days	Dietary selenium (mg/kg DM)			
		Group 0	Group 1	Group 2	Group 3
I[a]	1-14	0.20	0.31	0.21	0.21
II	15-70	0.07	0.17	0.20	0.29
III	71-147	0.08	0.42	0.45	0.78
IV	148-308	0.06	0.68	1.20	1.81
V	309-420	0.03	0.11	0.09	0.17
VI[b]	421-539	0.04	0.04	0.04	0.04

[a)] Standardization period, feeding was adjusted to meet requirement
[b)] Standardization period, no Se-supplementation

The purpose in the beef production trial was to compare the effect of inorganic sodium selenite and, through selenium fertilization, feed-incorporated selenium on selenium transport to tissues, effect on daily gain and health of the animals.

The calves for the beef production experiment were received from the cows involved in the milk production trial. They were reared on the same diets as their dams. The calves were in the experiment from the first day they were born until slaughtering at an average age of 14 months. The animals were fed individually. There were four groups in the experiment and each group included eight bulls. The selenium sources and levels in different groups were as follows:

Group	Diet	Dietary selenium (mg/kg DM)
0	Control	0.03
1	Sodium selenite	0.25
2	Selenized feed	0.25
3	Selenized feed	0.40

Selenized feed comprised selenium-enriched silage during the winter feeding period and selenized barley during the summer feeding period.

Results and discussion

Experiment with dairy cows

The intake of selenium by an animal depends not only on the selenium concentration in the feed but also on the feed consumption. The feed consumption did not differ greatly between the treatment groups. The average daily amounts of dry matter consumed varied between 17 and 20 kg/animal.

Milk production levels during the selenium supplementation periods (II-V) were 20.9, 23.3, 21.9 and 21.0 kg FCM/d in the groups 0, 1, 2 and 3, respectively. Only the production level in group 1 differed significantly from the other groups ($P<0.05$). The correlation between dietary selenium content and milk production was not significant. In many experiments it has been found that selenium supplementation does not increase milk production (Conrad & Moxon 1979; Gwazdauskas et al. 1979). Fisher et al. (1980), however, did find increase in milk production when selenium supplementation was fed to dairy cows, but their experiment was a short-term one lasting only 13 days. In our experiment the effect of selenium supplementation was the most positive during the first months of the lactation period (Fig. 1).

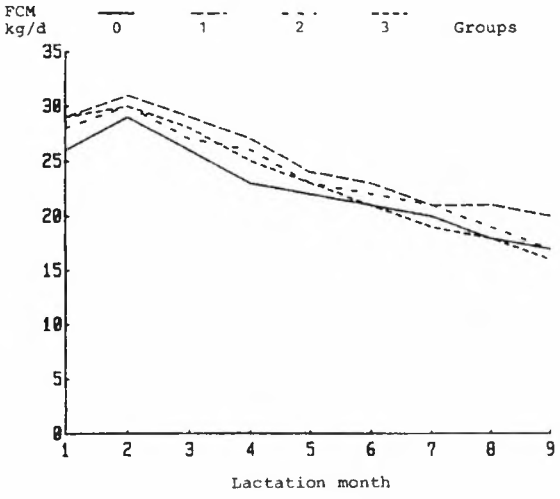

Fig. 1. Effect of selenium supplementation on milk production by cows

0 = control	0.03-0.08 mg Se/kg DM
1 = sodium selenite	0.11-0.68 mg Se/kg DM
2 = selenized feed	0.09-1.20 mg Se/kg DM
3 = sodium selenite + selenized feed	0.17-1.81 mg Se/kg DM

The selenium level in feed, 0.15 to 0.20 mg Se/kg DM, seemed to be sufficient in milk production. The highest selenium supplementation levels used in this experiment tended to decrease feed intake and, consequently, milk production.

Transfer of dietary selenium into milk was dependent on both selenium source and selenium content in the diet. During the standardization period, or before the experimental feeds were given, the level of selenium in the milk was the same in all the groups, 0.006-0.008 mg/kg (Table 2).

Selenium given in silage raised the selenium level of the milk to a greater extent than the same amount of selenium given in inorganic form. This is particularly clear-cut in periods II and III, in which the selenium concentration of the milk of the animals given selenium-treated silage (group 2) was twice as high as that of the milk of the animals receiving a corresponding amount of selenium as sodium selenite (group 1) (Table 2).

In the animals receiving selenium in silage the proportion of the selenium secreted into the milk averaged 8% (4-29%), whereas the corresponding values for the animal given sodium selenite was 5% (3-13%). The secretion percentage diminished as the selenium concentration in the feed increased. This occured with both sources of selenium.

Table 2. Transfer of feed selenium into milk

	Selenium content, mg/kg	
	Feed	Milk
Period II		
Group 0	0.070	0.008
Group 1	0.170	0.011
Group 2	0.200	0.021
Group 3	0.290	0.025
Period III		
Group 0	0.080	0.008
Group 1	0.420	0.016
Group 2	0.450	0.029
Group 3	0.780	0.034
Period IV		
Group 0	0.060	0.008
Group 1	0.680	0.020
Group 2	1.200	0.040
Group 3	1.810	0.051
Period V		
Group 0	0.030	0.008
Group 1	0.110	0.011
Group 2	0.090	0.023
Group 3	0.170	0.027

The results of these milk production trials are in accordance with those of other studies (Jakobsson et al. 1965; Conrad & Moxon 1979). When cows on a silage-grain diet were given a selenium supplement in the form of sodium selenite, less than 5% of the added selenium was transferred to the milk. When selenium was given in organic form bound to the mash, 19% of the selenium supplement was transferred to the milk (Conrad & Moxon 1979).

Experiment with growing cattle

The average daily gains of the animals were 982, 955, 1036 and 1036 g in the groups 0, 1, 2 and 3, respectively. Selenium supplementations had the positive effect on the daily gain, especially in the early stage of the experiment. During the first six months selenium supplementation at the 0.4 mg Se/kg feed DM level increased daily gain more than at the 0.25 mg Se/kg DM level. Later, the differences between the supplementation levels decreased and after six months selenium supplementation did not significantly ($P>0.05$) increase daily gain rates. Selenized feed increased growth rate more than sodium selenite.

In many experiments selenium supplementation did not have any effect on feed conversion rates (Pehrson & Johnsson 1985). However, there exist experiments where the effect has been positive (Byers & Moxon 1980). On the other hand, in some experiments selenium has worsened the feed conversion rate (Andersen et al. 1983). The worsened feed

conversion rate may be attributable to unpalatability of sodium selenite, which decreased feed intake. An improved feed conversion rate has been mainly reported during the early stage of the growing period (Byers & Moxon 1980). The protein content in diet has also affected selenium supplementation. It has been found that when dietary protein content is low, the effect of selenium supplementation is more positive than when the dietary protein content is high (Byers & Moxon 1980).

Selenized feed was more effective compared to sodium selenite in increasing selenium content in muscle tissues and liver, but not in kidney (for more detailed information see Anon 1987; Ekholm et al. 1991). Muscle selenium content also depended on dietary selenium content (Fig. 2).

SUPPLY OF SELENIUM IN BASIC CATTLE FEEDS IN FINLAND

As became evident above, the utilization of selenium by an animal depends on many factors. As the compounds in which selenium occurs also differ between the feeds, it is difficult to present an exact value for the Se requirements of domestic animals. This is also the reason why the dietary Se level reported to be sufficient in the literature varies widely, from 0.03 to 0.3 mg/kg feed DM. The concentration that may be considered to satisfy the average selenium requirement and to be safe as well is 0.1 mg/kg feed DM (Ammerman & Miller 1975). If the feed consists mainly of leguminous plants, however, ruminants should receive at least 0.2 mg/kg feed (Whanger et al. 1978).

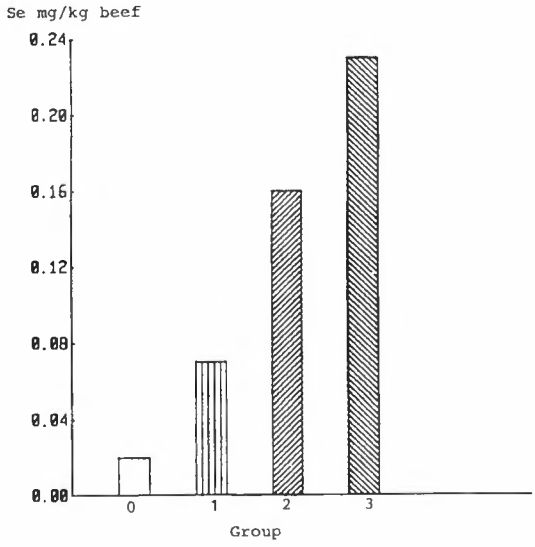

Fig. 2. Effect of selenium supplementation on beef selenium content (fresh weight)

0 = control	0.03 mg Se/kg DM
1 = sodium selenite	0.25 mg Se/kg DM
2 = selenized feed	0.25 mg Se/kg DM
3 = selenized feed	0.40 mg Se/kg DM

To satisfy the animals' Se requirements, the feeds should thus contain selenium at the level of 0.1 mg/kg DM. In Finland the Se content of the feed plants before Se-fertilization has been markedly below this level (Oksanen 1980). The selenium obtained from the basic feeds varied with the feeding alternatives and the production level from 10 to 60% of the selenium requirement. The selenium deficit was most often eliminated by adding selenium direct to the feeds. In Finland this has been allowed by law since 1969.

By supplementing the feeds with selenium, it has been possible to decrease the degenerative diseases caused in cattle by selenium deficiency, but not, however, to increase the selenium levels of the animal products to any great extent. This is partly due to the poor utilization of added inorganic selenium.

From the point of view of the human diet, it was desirable to obtain a further increase in the selenium levels of milk and meat, and this was the main reason for the decision to add selenium to fertilizers from 1984-85 onwards.

The addition of selenium to fertilizers (6 mg/kg) has raised the level of selenium in silage, hay and grain from 0.01-0.02 mg/kg DM to about 0.15-0.20 mg/kg DM. This means that cattle should receive their requirement of selenium from the basic feeds. The selenium concentration in milk has been increased from its 1983 level, 0.007 mg/kg fresh weight, to 0.015-0.020 mg and the corresponding increase in beef from 0.050 mg to 0.15-0.20 mg/kg fresh weight (Varo 1993).

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17 Effect of selenium on the health of the dairy cow, with special reference to udder health and reproduction

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This article is a literature review of the effects of selenium on udder health and reproduction in dairy cows. Some of the researchers referred to have noted very positive effects of selenium, but others have found no correlation between selenium and health parameters. According to the literature, recommended whole blood selenium concentrations range from 100 to 200 ng/ml.

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The traditional understanding has been that animal selenium supply can be at three different levels: deficient, sufficient and toxic. More recent research divides the intake into four levels: (1) deficient, (2) sufficient, (3) optimal, and (4) overoptimal. The division is based on animal immunological performance (Schmidt 1991). In transferring from areas of deficiency to areas of sufficient supply, traditional deficiency symptoms disappear, disease control is improved and sufficient intake initiates immunostimulation. Overoptimal supply, on the other hand, leads to immunosuppression (Larsen et al. 1988a, b).

EFFECT OF SELENIUM ON UDDER HEALTH

The positive effect of selenium on udder defence mechanisms has been reported in many studies. Boyne & Arthur (1981) observed a decrease in the ability of neutrophilic granulocytes to kill phagocyted *Candida albicans* cells in cows suffering from selenium deficiency. Lack of selenium also lowers the production of leucotrienes by polymorphonuclear leucocytes and thus the chemotaxis of neutrophils (Aziz & Klesius 1986). These observations are in agreement with those of the Grasso team (Grasso et al. 1990) concerning phagocytosis of neutrophils and bactericidal activity in cows suffering from selenium deficiency.

Selenium and vitamin E separately are sufficient to lessen the clinical symptoms of mastitis and to shorten their duration, but combined, they boost the positive effect (Smith et al. 1984). Selenium has been found to shorten the duration of mastitis and to alleviate

its symptoms, especially in *Escherichia coli* mastitis, but not in *Staphylococcus aureus* mastitis (Erskine et al. 1989, 1990).

Selenium and vitamin E supplementation decrease the prevalence of infections caused by environmental pathogens and staphylococcae during the calving period, and the incidence of clinical mastitis during the lactation of first-time calvers (Smith et al. 1985). Several studies on herd cell count and blood GSH-Px activity have yielded parallel results: a high cell count is connected with low GSH-Px activity and vice versa (Erskine et al. 1987; Weiss et al. 1990a; Braun et al. 1991).

EFFECT OF SELENIUM ON REPRODUCTION

In studying the connection between selenium and reproduction, researchers originally focused mainly on the retained placenta. Julien et al. (1976) obtained excellent results after adding selenium to feed during the dry period. Since then, there have been studies that support the positive results (Ishak et al. 1983; Braun et al. 1991), cannot confirm the positive results (Gwazdauskas et al. 1979) or can only partially confirm them (Segerson et al. 1981). Larson et al. (1980) found a positive relationship between serum selenium levels and success in insemination, a result in agreement with that of Segerson et al. (1977). On the other hand, no difference was found between selenium levels in herds of different fertility levels (Braun et al. 1991).

Harrison (1984) obtained promising results in the ability of selenium injections to prevent cystae and metritis during the dry period. Adding vitamin E to the injection also decreased the incidence of retained placenta, while in a Norwegian study, no correlation was found between selenium balance and reproduction parameters (Ropstad et al. 1987).

RECOMMENDED BLOOD SELENIUM CONCENTRATION

In whole blood, 100 ng/ml is considered a sufficient selenium concentration, concentrations of under 50 ng/ml can be considered deficient (Koller et al. 1983). According to a US mastitis investigation group, the lowest limit of sufficient intake in preventing udder infections is 200 ng/ml of whole blood and 70 ng/ml of serum (Smith et al. 1988), which is higher than earlier recommendations for serum (Stewens et al. 1985).

PRELIMINARY RESULTS OF THIS STUDY

The recommended whole blood concentration of 100 ng/ml was exceeded in almost all cows in the research material (Koller et al. 1983). The dispersion of measured whole blood selenium values was considerable. Selenium supplementation is not necessary for achieving the set recommendation level. The highest values were so high that random inorganic selenium supplementation may cause immunosuppression. The limit of 200 ng/ml set to eliminate udder infections (Smith et al. 1988) was exceeded only in animals with above-average concentrations. The herd was found to be a major reason for selenium value

dispersion. Therefore, no recommendation can be given for selenium supplementation levels without knowledge of the herd selenium levels.

SUMMARY

The relationship between selenium and cow health parameters is studied either *in vitro* or on live animals using experimental or field techiques. Furthermore, the selenium supply of experiment and control groups has not been on the same level in all studies. These factors cause most of the apparent discrepancies in the results. Even so, the positive effect of selenium on the immunological competence of the dairy cow cannot be questioned.

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18 Effects of selenium fertilization on the human selenium status and the environment

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In Finland the serum selenium concentration of healthy subjects was 0.89 μmol/l and that of toenails 0.47 mg/kg before selenium fertilization was introduced in 1985. The selenium fertilization programme increased the selenium levels by 56% and 104% in serum and toenails, respectively, and also raised the serum selenium concentration to a similar extent in populations at risk of low intake. This was most probably due to the wide distribution of selenium in different food groups. The mean selenium concentration in lake water (n=31) was 62 ng/l. Leaching of soluble selenium from fields to lake waters was not detected. The large variation in selenium concentrations in tap-water (15-1000 ng/l), mostly groundwaters, is mainly a reflection of the local high-selenium bedrock.

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THE HUMAN SELENIUM STATUS

The primary purpose of adding selenium to fertilizers in Finland was to raise the selenium concentration in foodstuffs. This measure resulted in a threefold increase in selenium intake (Varo 1993). The selenium intake of individuals varies considerably from day to day as well as between individuals (Mutanen 1984). Determining tissue selenium levels can decrease these variations considerably, although levels are also susceptible to various physiological parameters, such as chemical species of selenium, intestinal absorption, age, etc.

Different tissues reflect the intake of selenium on a different time-scale. Plasma reflects previous intake over a time period of approximately several weeks, whole blood over one to two months and, finally, nails over a period of six to twelve months (Longnecker et al. 1993). In selenium supplementation studies performed in Finland, organic selenium in the form of selenium-rich yeast or wheat flour resulted in a steady-state within 11 weeks (Levander et al. 1983; Alfthan et al. 1991a).

Follow-up of the selenium status during the selenium fertilization was of importance in order to detect possible effects of unduly high selenium intakes, but also to screen for eventual low levels of tissue selenium in individuals.

Healthy adults

Follow-up of the serum selenium concentration in healthy adults was initiated in 1983, two years before the fertilization with selenium was started (Alfthan 1988) and has continued ever since. Serum samples from the same 24 subjects of the urban follow-up group had been freeze-stored from 1975 and were available for selenium analyses. Figure 1. shows that in the 1970s the mean serum selenium concentration varied between 0.63 and 0.76 μmol/l[1] when the intake depended solely on domestic grains (Mutanen & Koivistoinen 1980). In the 1980s importation of high-selenium grain raised the intake, which was reflected in peak serum selenium levels in 1979, 1980 and 1982 (Alfthan 1988). Selenium fertilization in 1985 increased the mean serum selenium level of healthy adults gradually from 0.89 μmol/l in 1984 to a new level of 1.52 μmol/l in 1989-90, (See Fig.1) (Varo et al. 1993 in press). This gradual response is due solely to the trend in the intake.

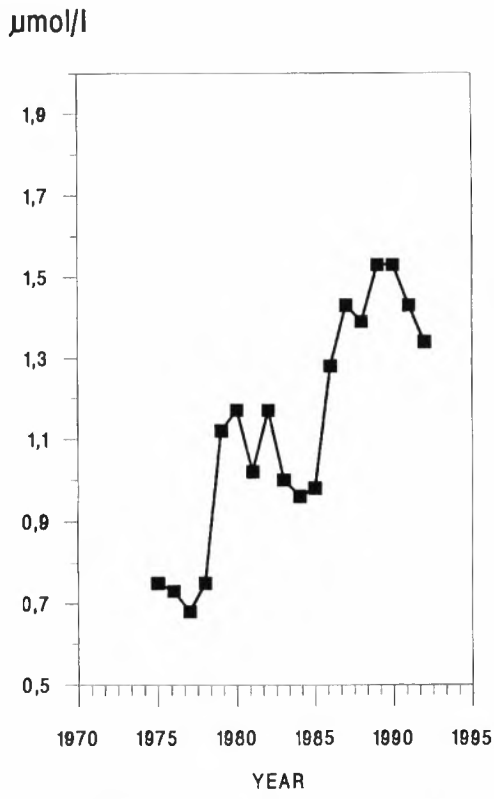

Fig. 1. Serum selenium concentration of adults in Finland before and during selenium fertilization

Selenium levels reported from various countries in Europe range from 0.70 to 1.65 umol/l (Thorling et al. 1986; Lockitch 1989). The lowest levels have been found in Hungary (Alfthan et al. 1992a) and the highest in Norway (Lockitch 1989). The serum selenium level during the peak years 1989-90 was second highest in Europe. However, since 1991 the amount of selenium in fertilizers for human crops was decreased from 16 to 6 mg/kg. Preliminary data indicate that the serum selenium level has decreased below 1.27 μmol/l.

Neonates, pregnant women and the elderly

The serum selenium concentration of sub-populations thought to be at risk of selenium deficiency has been followed since early 1980s. Among these the increased intake of selenium increased the levels of newborns by 52% and of pregnant women by 63% (Fig. 2) (Varo et al. 1993 in press).

The serum selenium concentration of two cohorts of elderly men was determined in 1984 before selenium fertilization (Kivelä et al. 1989) and again during 1989 (Pekkanen et

[1] To convert μg/l to μmol/l divide by 78.9

al. 1993, personal communication) after selenium fertilization. Initially, the level was 0.92 μmol/l, not significantly different from that of healthy middle-aged subjects (Varo et al. 1993 in press). Because of the increase in intake the mean serum selenium level increased by 42% to a level comparable with middle-aged adults. Neither extremely low nor high individual values were found, range 0.88-2.5 μmol/l.

Institutionalized elderly female patients (mean age 83 years) had a mean serum selenium concentration of 0.67 μmol/l before the onset of fertilization (Alfthan et al. 1988), a level that was significantly lower than that of middle-aged healthy females (see Fig. 3). Three years after the onset of fertilization the selenium level of the elderly patients had increased by 104% and was no longer different from the mean of the younger women.

This lack of difference suggests that although the energy intake of these patients was low, an adequate intake of selenium was provided because of the wide distribution of selenium between milk products, meat and cereals (Varo 1993).

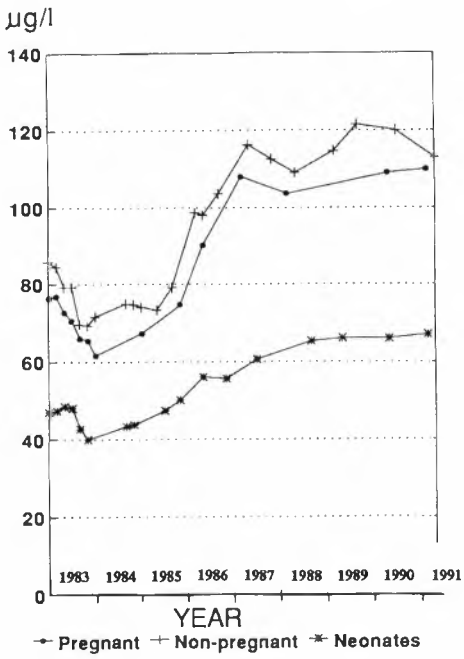

Fig. 2. Serum selenium concentration of healthy middle-aged women, pregnant women and neonantes in Finland before and during selenium fertilization.

Toenail selenium

Toenails are a long-term indicator of selenium intake (Morris et al. 1983; Longnecker et al. 1993). Geographical differences in selenium intake were clearly seen in toenail selenium levels. Before selenium fertilization the toenail selenium concentration in Finland, 0.47 mg/kg (Ovaskainen et al. 1993), was comparable to levels reported from New Zealand (Morris et al. 1983), a low-selenium country (Fig.4).

The increased intake was also reflected in the toenail selenium level in 1988 in a comparable group of healthy males (Alfthan et al. 1991b). By 1991 in a study on farmers (Alfthan et al. 1992b), the level had reached a mean value of 0.96 mg/kg.

There is only limited data on the toenail

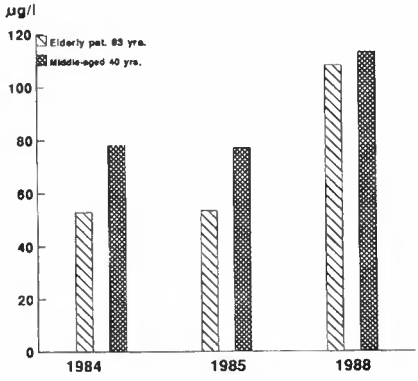

Fig. 3. Serum selenium concentration of healty middleaged women and institutionalized elderly female patients.
To convert μg/l to μmol/l divide by 78.9

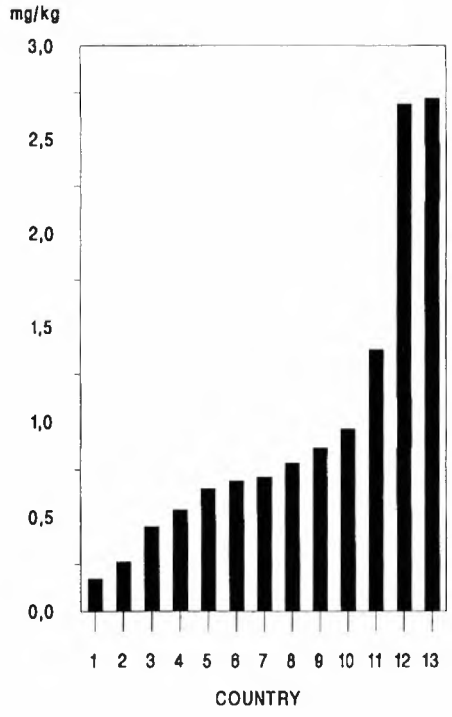

Fig. 4. Toenail selenium concentrations in various countries. Numbers refer to country and reference:

1. China, Sichuan, Yang et al. 1987
2. New Zealand, Morris et al. 1983
3. Finland 1984, Ovaskainen et al. 1993
4. Hungary, Alfthan et al. 1992
5. Netherlands, van't Veer et al. 1990
6. Finland 1988, Alfthan et al. 1991a
7. China, Enshi, Yang et al. 1989
8. Netherlands, Rotterdam, Kok et al. 1989
9. Finland 1991, Alfthan et al. 1992a
10. USA, Washington, Rogers et al. 1991
11. USA, S. Dakota, Longnecker et al. 1993
12. Venezuela, Villa Bruzual, Negretti de Brätter et al. subm.
13. China, Enshi, Yang et al. 1989

selenium of healthy adults from other countries (see Fig. 4). Compared with the mean level of a healthy population sample from the US Midwest (Longnecker et al. 1993), where no adverse effects as a result of high dietary intake of selenium have been reported, the highest mean level occurring in Finland in 1991 was still only 62% of the US Midwest mean level.

ENVIRONMENTAL STUDIES

Selenates are readily soluble salts. Public and professional concern has been expressed on the fate of sodium selenate added to fertilizers and applied to fields throughout the country. The selenium concentration of different types of natural waters has been monitored regularly since before the introduction of selenium fertilization (Ministry Report 1990), but selenium levels below 1000 ng/l could not be detected by the analytical method. In samples taken up to 1989 no selenium could be detected exceeding the detection limit of the method. This was satisfactory from a public hygiene point of view, as the regulatory limit for selenium set for drinking-water is 10 000 ng/l in Finland, but it was not adequate to detect changes in absolute selenium concentrations of natural waters.

A series of studies was intitiated in 1989 by the National Public Health Institute in cooperation with the National Board of Waters and Environment to elucidate the effects of selenium fertilization on waters.

Lakes

The mean $\pm$ SD selenium concentration in lakewaters sampled in 1990 was (n=31) 62 $\pm$ 18 ng/l ranging from 25 to 114 ng/l (Wang et al. 1991). Although the sampling was undertaken five years after the selenium fertilization started, careful selection of the lakes overcame this bias. The mean selenium concentration in water from lakes heavily affected by agriculture, 64 ng/l, n=13, did not differ significantly from lakes in natural surroundings, 60 ng/l, n=18.

Tap-water

The first survey on selenium in tap-water sampled in 1990 in 21 towns in Finland demonstrated the difficulties encountered in trying to relate the effects of selenium fertilization on water (Wang et al. 1991). The mean selenium concentration was 167 ng/l with a range of 13 to 1103 ng/l. There was no apparent association between tap-water selenium concentration and geographical location or regarding agricultural activity.

Preliminary data suggest that one of the major factors explaining the selenium concentration in groundwaters is the very locally occurring selenium-rich shales of the bedrock (Koljonen 1975). The tap-waters with the highest levels of selenium were either natural groundwater or man-made groundwaters filtered through morainic ridges (Wang D, personal communication).

Rivers

The selenium concentration of rivers from southern Finland sampled within a 50 km radius from Helsinki in 1990 (n=21) ranged from 43 to 220 ng/l, with a mean value of 85 ng/l (Wang et al. 1991). The lowest mean level of selenium (56 ng/l) was found in the four rivers of the western coastal area, the area most heavily affected by agriculture.

The effect of melt-water from snow was studied by repeated sampling of six rivers before and after the melting of snow (Wang et al. 1991). Washout of selenium from fields in the catchment area of the rivers did not increase, but, on the contrary, decreased the river water selenium concentration by a mean 25% (range 19 to 39%). This suggests that selenium was not leached into the waters from the fields fertilized with selenium. On the other hand, the mean selenium concentration of snow was 91 ng/l, which, if able to leach selenium from the soils, would have increased the selenium concentration of at least the low-selenium rivers.

CONCLUSION

In conclusion, the selenium concentration in serum and toenails of healthy subjects has increased by 56% and 104%, respectively, as a result of the selenium fertilization. The selenium fertilization raised the serum selenium concentration also of populations at risk of low intake to a similar extent. This is most probably due to the wide distribution of selenium in different food groups.

Leaching of soluble selenium from fields to lake waters was not detected. The large variation in levels of selenium in tap-water (mostly groundwaters) is mainly a reflection of the local high-selenium bedrock.

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19 Optimization of the selenium flow to man

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Edelmann, K. 1993. Optimization of the selenium flow to man. Norwegian Journal of Agricultural Sciences. Suppl. No.11: 183-187. ISSN 0802-1600.

PRESENT STATUS IN FINLAND

In many territories the local crop supplies insufficient levels of selenium into the nutrition chain. In order to elevate the organic natural selenium content of the human diet, it has been suggested that commercial fertilizers be fortified with inorganic selenate, a policy that has been introduced in Finland since 1985. This, as has been reported, has increased the average selenium intake of an average Finn to an acceptable level, viz. about 100 mcg/d. (Fig. 1)

THE CHEMISTRY OF SELENIUM IN THE SOIL WAS UNEXPECTED

When the selenium fertilization experiment was begun in Finland, it was supposed that selenate, when used as a selenium source in the acid soils of Finland, would soon be reduced into selenite, which in turn would soon be bound to heavy metals as insoluble complexes (Koivistoinen 1986). Since then it has been proposed that selenate remains soluble in alkaline soils and can become soluble anew when conditions have changed (Vuori et al. 1989). Moreover, selenate is regarded as the environmentally most dangerous form of selenium (WHO 1987).

The results of the Finnish experiment also show that the selenium yield in crops varies greatly (Selenium Monitoring Group of the Ministry of Agriculture and Forestry of Finland 1991), (Table 1). This and the fact that the increase in the selenium content in autumn wheat and rye is low (Table 2), might be attributable to the leaching of selenate into the surface waters rather than selenium building insoluble complexes with heavy metals as suggested.

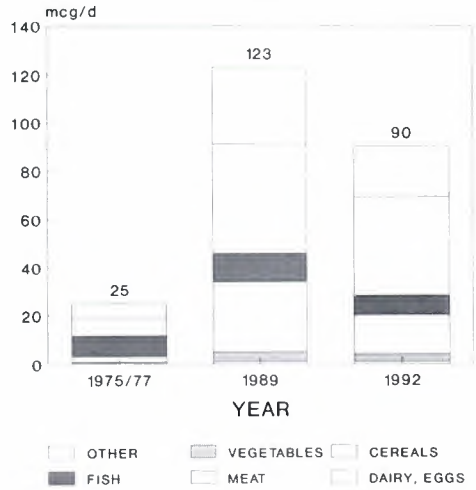

Fig. 1. Selenium intake in Finland. Energy level 10 MJ. Source: Ministry of Agriculture and Forestry. Report of the Selenium Monitoring Group 1992 Preliminary values

Table 2. The selenium contents of barley and oat grain (mg/kg of dry matter
Source: Report of the Selenium Monitoring Group 1991

Year	n	Mean	Range
Barley			
1984	50	0.008	0.004 - 0.016
1985	240	0.16	0.007 - 0.56
1986	211	0.21	0.013 - 0.58
1987	177	0.15	0.013 - 0.40
1988	100	0.23	0.002 - 0.62
1989	100	0.23	0.010 - 0.51
Oat			
1984	49	0.009	0.004 - 0.015
1985	200	0.15	0.008 - 0.41
1986	216	0.20	0.005 - 0.52
1987	171	0.13	0.020 - 0.45
1988	100	0.26	0.036 - 0.84
1989	100	0.23	0.006 - 0.75
Target level		0.1	

Table 3. Selenium content of Finnish food grain (mg/kg of dry matter). Every sample represents 50,000 - 500,000 kg grain
Source: Report of the Selenium Monitoring Group 1991

Year	n	x+SD		Range
1984				
spring wheat	12	0.012	0.007	0.004 - 0.026
rye	10	0.009	0.003	0.006 - 0.015
1985				
spring wheat	17	0.225	0.097	0.076 - 0.367
autumn wheat	4	0.026	0.018	0.012 - 0.052
rye	9	0.023	0.018	0.003 - 0.066
1990				
spring wheat	24	0.280	0.077	0.150 - 0.485
autumn wheat	22	0.069	0.038	0.025 - 0.183
rye	23	0.068	0.033	0.026 - 0.148
Target level		0.1		

It has been shown that selenium is essential in the metabolism of bacterioplankton (Eriksson 1990). Simultaneously with the beginning of the selenium fertilization the discovery of blue-green algae has increased in Finland (Lepistö 1988), (Fig. 2).

DAIRY PRODUCTS, EGGS AND MEAT - MOST IMPORTANT SELENIUM SOURCES IN DIET

In 1989, as a result of the selenium fertilization experiment, over 60% of the average selenium intake in Finland came from meat, dairy products and eggs. In the "low selenium years", 1975/77, the proportion was 53%.

The increase in the content of organic-bound selenium in Finnish feed as a result of fertilization is estimated to be about 0.15 ppm Se in DM. All commercial feed has been supplemented in Finland since 1969 with 0.1 ppm selenium as selenite. This has not, however, had a significant effect on the selenium content in meat and milk.

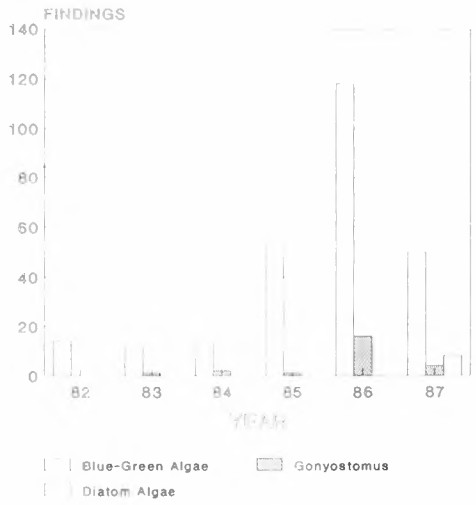

Fig. 2. Findings of algae in Finland as reported to the authorieties.
Source: Liisa Lepistö: Studies on Algae by Water Authorities; Alarming Changes in Water-ecosystems; Helsinki 1988, p. 95

SELENIUM YEAST IN FEED INCREASES SELENIUM CONTENT IN MEAT AND MILK

Since the early 1980s we have produced selenium yeast for human and animal consumption. This standardized product, which contains 500 ppm organic-bound selenium, has been used in numerous growth and bioavailability tests showing expected positive effects.

For animal feed the product has a trade mark ALKOSEL. Using this as a feed ingredient, the producer will derive a considerable economic benefit from the improved growth and health status of animals.

In order to clarify the exact flow of selenium from yeast to food products via animals, we have recently started a study in Estonia with cows. The aim of this study is to compare the bioavailability of selenite and ALKOSEL as to the effect on the selenium content in milk and meat.

So far the results unambiguously show that ALKOSEL raises the selenium content in milk very effectively, whereas selenite has no significant effect (Fig. 3).

SELENIUM YEAST AS A SOURCE OF ORGANIC SELENIUM

Supplementing feed with about 0.15 ppm Se from ALKOSEL will result in the same selenium intake in man as has been received in the Finnish fertilization experiment. This would mean that instead of using 11,000 kg selenate selenium a year, less than a tenth of the amount of selenium would be enough to reach the same result.

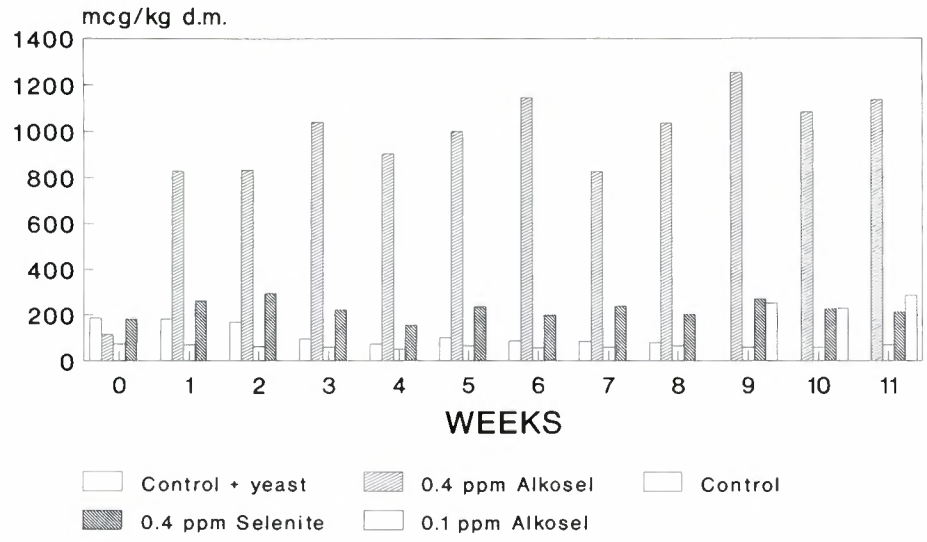

Fig. 3. Supplementation of feed with alcosel vs selenite
Source: Trial with milking cows 1993. Four animals in each group

Yeast is used as the first biological filter instead of plants. The flow of selenium is well controlled, and with no fear of unexpected environmental risks (Fig. 4).

As selenium containing baker's yeast can be used for baking, the selenium intake through bread can also be adjusted, when needed.

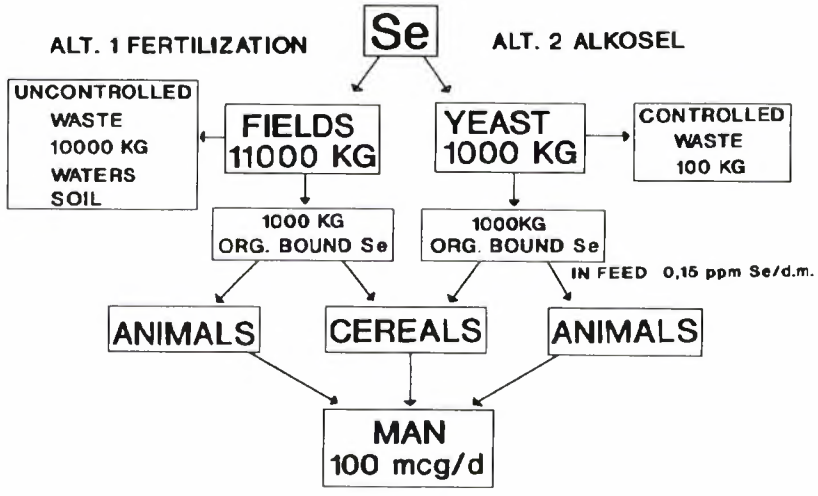

Fig. 4. Se in nutrition chain annually for five million people
Source: Alko Biotechnology

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20 Selenium supplementation in pigs

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Since the identification of selenium (Se) as an essential nutrient a great deal of effort has gone into defining the function of Se and into characterizing the magnitude of the Se-deficiency problem. In particular this topic has been investigated in the Scandinavian countries, where the soil has a low Se content. Furthermore, many research groups have studied different approaches to overcome the Se-deficiency associated problems. One way has been to supplement the diets for domestic animals with Se, and in order to estimate how much Se should be added to the diets, it is necessary to know the requirements of pigs for this nutrient. Consequently, many experiments have been carried out to study the effects of supplementing pig diets with Se. This review describes recent data concerning the effects of supplementing different levels (ranging from suboptimal, over subtoxic to toxic levels) to pig diets on performance as well as on physiological characteristics.

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Since the identification of selenium (Se) as an essential nutrient by Schwarz & Foltz in 1957 considerable effort has gone into defining the function of Se and into characterizing the magnitude of the Se-deficiency problem. Although Se is widely distributed in the earth's crust, concentrations and availability in soil are extremely variable. Thus, there are regions where Se supplementation must be used to ensure that animals remain healthy and productive. Since the late 1950s many research groups have concentrated their efforts in establishing the dietary amount necessary to fulfil the Se requirement in domestic animals.

Until 1974 it was illegal in the United States to supplement diets for pigs with Se (Ullrey 1992). This year it was approved that supplements of Se from sodium selenite or sodium selenate should not exceed 0.1 ppm for pigs. Later, in 1982, supplements up to 0.3 ppm in prestarter and starter diets for pigs were accepted, and in 1987 a general increase in the maximum level of supplemental Se from 0.1 to 0.3 ppm in complete feeds for all major food-producing animals was approved (Ullrey 1992). As soils in the Scandinavian countries generally have a low Se content, this appears to be responsible for the Se-deficiency disorders reported in livestock raised in this region. This resulted in demands for a legislation for Se supplementation, and in 1969 Finland was the first Scandinavian country to approve Se supplementation to pig diets, whereas it was not until 1980 that Norway and Sweden legalized Se supplementation. In 1975 a supplementation of 0.1 ppm Se to pig diets was approved in Denmark, but in 1991 the recommendation was changed to 0.35 mg/FUp (about 0.4 ppm) for prestarter and starter diets and 0.2 mg/FUp (about 0.22 ppm) for both growing and breeding pigs. The general maximum accepted total dietary Se level in Denmark is 0.5 ppm.

Although most Se-deficiency disorders have disappeared, there is still much discussion and work being carried out concerning the requirement of Se, and how much Se and in which form it should be supplemented to the diets for pigs. Contradictory results have been obtained, and this inconsistency is probably due to several factors, among which are the dietary vitamin E content and the choice of response characteristics. Consequently, Se recommendations vary among countries and are rather imprecise. The National Research Council (NRC) (1988) states that the dietary requirement for Se is between 0.1 and 0.3 ppm, whereas The Agricultural Research Council (ARC) (1981) tentatively proposes a requirement for growing pigs up to 90 kg of 0.16 ppm Se. These recommendations will generally require some Se supplementation to pig diets.

WHICH CRITERIA SHOULD BE USED IN INVESTIGATING THE NEED FOR SUPPLEMENTING SELENIUM TO PIG DIETS?

In establishing the Se requirement for pigs, several factors are important, such as for instance performance, health, Se concentration and GSH-Px activity in the blood (plasma, serum, erythrocytes, or whole blood), and perhaps the immune response. As the metabolism of Se depends on its chemical form, it is important to be aware of which Se source is in question. A third factor to take into consideration is the age and physiological stage of the pigs.

From a practical point of view performance traits are important, whereas the underlying characteristics such as Se concentrations and GSH-Px activities seem more of scientific interest or are only useful in practical situations when problems arise. From a physiological point of view, the basic functions of Se are of more interest, whereas maximizing performance traits is rather a result of fulfilling the basic functions of Se. Consequently, the establishment of an exact Se requirement in pigs is difficult and clearly depends on the choice of physiological characteristics, which justifies the vague NRC statement that the dietary requirement for Se lies between 0.1 and 0.3 ppm in pigs.

EFFECTS OF Se SUPPLEMENTATION TO PIG DIETS

It is generally accepted that Se supplementation results in increased Se concentration and glutathione peroxidase (GSH-Px) activities in tissues and blood. Furthermore, many studies have revealed correlations between the dietary Se content and Se concentration and GSH-Px activity in blood and tissues. Therefore, this topic will not be dealt with in this paper, but other aspects concerning Se supplementation in pigs with special emphasis on experiments of recent date will primarily be reviewed.

Sows

In a trial with sows fed a diet with an intrinsic Se content of 0.1 ppm supplemented with either 0, 0.4, or 0.8 ppm Se Neumann & Bronsch (1988) found that the GSH-Px activity was highest and the number of stillborn pigs was lowest when sows were fed 0.5 ppm Se. They concluded that the requirement of the sow is met at a level of 0.5 ppm.

A Danish study comparing an unsupplemented (0.13 ppm) diet with a sodium selenite supplemented (0.24 ppm) diet for sows for four parities revealed that supplementation increased litter size at birth and at weaning and decreased the mortality among piglets in the sucking period. But the number of stillborn piglets, body weight of the piglets, milk yield, and weaning to oestrus interval were unaffected by Se supplementation (Poulsen, data to be published).

As expected, the Se concentration in blood in this study was highest in Se supplemented sows, leading to an increased GSH-Px activity. There was a time-lag between the effect on Se concentration and GSH-Px activity caused by the relatively long lifetime of the erythrocytes. In contrast to the unsupplemented sows, the Se concentration in the Se-supplemented group was almost constant during all four parities, while the GSH-Px activity varied through each parity in both sow groups. In general, the activity was highest at the beginning of each pregnancy and lowest during the lactational period.

The Se concentration in liver from newborn piglets was not affected by adding Se to the sows' diet, revealing that the size of the Se pool in foetuses was not increased when Se was added to the sows' diet during pregnancy. Generally, the Se concentration in liver was higher in two week-old piglets than in newborn piglets. But because of an increased Se content in the sows' milk, the Se concentration in liver was 50% higher in piglets sucking Se-supplemented sows. The GSH-Px activity in liver from newborn as well as two week-old piglets was highest after the sows' diets were supplemented with Se. The obtained results indicate that a dietary level of 0.13 ppm did not fulfil the sows' Se needs, which probably were met at a dietary level of 0.24 ppm.

In an experiment conducted on commercial herds Jensen et al. (1984) found that intramuscular injection of sodium selenite to sows and gilts three to six weeks before farrowing did not affect either GSH-Px in whole blood or the number of liveborn, stillborn, and weaned piglets. Furthermore, the number of days between weaning and oestrus was not affected. Chavez & Patton (1988) suggested that vitamin E and Se supplementation by periodic injections may result in increased litter size at birth and at weaning, as well as an increase in litter weight at birth. As the dietary Se level was 0.1 ppm, they suggested that a dietary Se supplementation above this level might improve the reproductive performance of sows. This was confirmed by the above-mentioned Danish experiment concerning supplementation of Se to sows.

Piglets and growing pigs

The placental transport of Se from sows to their foetuses, measured as Se concentration in serum, increased when the dietary Se supplementation to sows was increased (0, 0.1, and 0.5 ppm) (Mahan et al. 1977). The authors registered a slight decline in Se concentration in serum in piglets postpartum, whereas the Se content, although affected by treatment, was constant during the sucking period. At weaning there was a drop in the Se concentration in serum in piglets from all treatment groups, which were fed the same Se-deficient (0.1 ppm) diet after weaning. Mahan et al. (1977) concluded that the carry-over from the sow is of a relatively short duration, such that within a week or two after weaning the piglet is dependent upon its diet to meet its need for Se. In another study, Mahan (1985) found that the Se retention was linear when 0 to 1.0 ppm Se was added to the diet for weaned pigs. By comparing 0.1, 0.3, 0.5, or 0.7 ppm dietary Se to weaned piglets, Mahan (1987)

concluded that the requirement may be higher than 0.3 during the initial 14 days post-weaning, but from 14 to 35 days the dietary Se content of 0.3 was adequate.

From the above-mentioned Danish experiment with sows, five piglets per sow from parity four were at weaning allotted to another experiment in order to investigate the effect of supplementing 0, 0.1, 0.2, 0.3, or 0.4 ppm Se as sodium selenite to a diet containing 0.13 ppm intrinsic Se. During the post-weaning period (4 to 10 weeks of age) there was no effect of Se supplementation on performance, and the same was valid for the last period (10 weeks of age until slaughter). But measured over the whole period there was a significant linear and quadratic effect of Se supplementation on daily gain (Poulsen, data to be published). The meat percentage at slaughter was unaffected by treatment, but the Se content in the loin muscle was significantly lower in the unsupplemented pigs. Among the sodium selenite supplemented pigs, increased Se supplementation did not affect Se concentration in the muscle. At weaning the GSH-Px activity in blood was highest in pigs sucking sows fed the Se supplemented diet, but at 10 weeks of age no sow effect could be measured. Contrary to this, there was a clear effect of dietary Se supply, so that the GSH-Px activity in pigs fed the unsupplemented diet decreased, while Se addition resulted in an increased GSH-Px activity. There were only minor differences between Se-supplemented groups. These results indicate that a dietary Se level between 0.3 and 0.4 ppm is necessary to fulfil the need in weaned pigs, whereas because of a higher daily intake, the need in ppm in finishing pigs is lower. Furthermore, the experiment revealed that a higher total dietary Se level than 0.5 ppm may be harmful and may result in lower daily gain, when Se is supplemented as sodium selenite.

MULBERRY HEART DISEASE - SELENIUM DEFICIENCY OR NOT?

Although supplementation of Se as well as vitamin E is commonly used, mulberry heart disease (MHD) still persists among young pigs. Pigs diagnosed as having MHD compared with control pigs showed no difference in Se concentration in liver and heart tissues postmortem, indicating that Se deficiency is not of aetiologic interest, and that this disease probably has a hereditary aetiology (Vasa 1986; Nielsen et al. 1989; Kennedy et al. 1989). Recently, Korpela (1990a, b) reported that the myocardial and hepatic iron concentration was increased in pigs with mulberry heart disease (microangiopathy), which might have promoted oxidative stress in Se-vitamin E deficient pigs and thus contributed to the development of oxidative stress.

IS THE EFFECT OF SELENIUM SUPPLEMENTATION UNDER GENETIC CONTROL?

The sporadic nature of MHD suggests that some pigs may have an impaired, perhaps genetically influenced, ability to utilize dietary Se. Jørgensen et al. (1977) alluded to this hypothesis, and it is consistent with several later studies reporting not only the Se supplemental effect but also a genetic influence on GSH-Px. Stowe & Miller (1985) investigated the effect of adding either 0.1 or 0.3 ppm Se to the diets of selected hypo-selenemic or hyper-

selenemic pigs. They found a significant difference in mean serum Se concentration of selected hypo- or hyper-Se pigs fed 0.1 ppm Se, whereas the increase in serum Se attributable to 0.3 ppm supplemental dietary Se was greater among the selected hypo-Se pigs than among the hyper-Se pigs. The selected hyper-Se pigs maintained a more rapid growth rate than hypo-Se pigs. Balance trials showed that hypo-Se pigs excreted 8.3% more Se in the faeces, excreted 1.7% less Se in urine and retained 6.6% less Se than the hyper-Se pigs, indicating that there is a genetic influence on Se absorption in the pig (Stowe & Miller 1986).

These results demonstrate that it is important to take heredity into consideration when studies concerning Se supplementation are carried out. Lingaas et al. (1991) found that the heritabilities for Se concentration and GSH-Px activity in pig plasma were 0.41 and 0.47, respectively, and thus they confirmed that it is possible to increase the levels of both Se and GSH-Px in pig plasma by selection. These results also indicate that in low Se areas natural selection may have taken place in order to maximize Se retention in domestic animals as well as human beings (Lingaas et al. 1992).

IMMUNE RESPONSE

In a study with four daily supplemental Se levels of 0, 0.2, 0.4, or 0.8 mg per pig, Teige et al. (1984) investigated the effect of supplementation on *Treponema hyodysenteriae* inoculated pigs. The best protection was found in pigs given 0.4 mg Se per day.

The immuno competence was also investigated in offspring from sows fed a dietary Se level of 0.13 ppm supplemented with 0, 2, 4, 8, or 16 ppm Se as sodium selenite. The best humoral immune response was found, when the Se supplementation was 2 ppm, while the subtoxic and toxic levels resulted in a decreased response (Kruse & Poulsen 1989).

Blodgett et al. (1989) comparing addition of 0.1 or 0.9 ppm Se to sows, found no immunostimulatory effects of supplemental Se on colostric Ig concentration or passive antibody titers in newborn pigs. In earlier work with weanling pigs, the humoral response to novel antigens seemed to favour diets supplemented with 0.9 compared with 0.3 ppm Se (Blodgett et al. 1988).

These studies indicate that maximizing the immuno competence might require a higher dietary content of Se than that needed for maximizing performance. Further studies are needed to illustrate this.

TOXICITY

Dietary Se concentrations far above requirement level resulted, as expected, in increased blood Se concentration and GSH-Px activity (Goehring et al. 1984a, b). But Goehring et al. (1984b) stated that the most sensitive indicator of chronic selenosis in growing pigs was growth rate. Mahan & Moxon (1984) concluded that a dietary Se level of 2.5 ppm did not depress post-weaning performance in piglets whereas 5.0 ppm or higher depressed performance and produced the classical symptoms of selenosis. The above-mentioned Danish study with piglets and growing pigs showed a reduction in daily gain at as low a dietary Se

level as 0.5 ppm (Poulsen, data to be published).

Studying the effect of excessive dietary selenium in primiparous sows, Poulsen et al. (1989) found that the addition of 0 to 16 ppm Se as sodium selenite to feed for sows and their piglets up to nine weeks of age did not cause any manifest toxic effects. The reproductive performance of the sows did not differ among treatment groups, and the piglets were all fullborn. The litter weight at birth was unaffected, while piglets fed 8 or 16 ppm Se had significantly lower body weight at nine weeks of age. Above 2 ppm dietary Se, GSH-Px activity in blood was found not to be correlated to Se intake.

These studies confirm the toxicity of Se. Above a dietary level of 5 to 6 ppm the Se surplus gradually becomes more serious, resulting in a pronounced reduction in growth and, later, in the classical symptoms such as lesions in the central nervous system.

CAN SUPPLEMENTAL SELENIUM PREVENT STRESS RELATED MYOPATHY IN HALOTHANE-POSITIVE PIGS?

It has been proposed that Se supplementation could prevent stress-related myopathy in halothane-positive pigs, but Hänichen et al. (1988) found that the supplementation of either 0.1 or 0.5 ppm Se to diets for halothane-positive pigs had no influence on this stress-related myopathy. Furthermore, Wiegand et al. (1984) found that the erythrocytic GSH-Px activity recorded from halothane-positive pigs was only negligibly higher compared to halothane-negative pigs.

SELENIUM SOURCES

Although this subject is outside the scope of this review and will be covered by others, some general remarks will be given. Clearly, deviations in the metabolism of selenium from inorganic and organic sources result in different responses. Therefore, in comparing the effects and "quality" of different Se sources, the choice of response traits is of great importance. Many studies have been conducted to characterize these effects of different Se sources on Se concentration and GSH-Px in blood and tissues and, to a lesser extent, on performance.

Recently, Mahan & Magee (1991) compared the effects of calcium selenite or sodium selenite at three different levels (0.3, 5, or 15 ppm Se). They found that calcium selenite was as effective as sodium selenite using the measurement criteria of growth, serum and tissue Se concentrations, and GSH-Px activity in growing pigs. No difference in performance measurements was observed between 0.3 and 5 ppm Se, but when the 15 ppm of Se was provided the performance declined when Se from either source was supplied.

In a study with growing pigs Suomi & Alaviuhkola (1992) found no difference in performance, carcass quality, Se content in liver and serum between pigs fed diets supplemented with 0.1 ppm inorganic Se (selenodioxid), 0.1 ppm organic Se (yeast), or 0.4 ppm organic Se (yeast). Compared with 0.1 ppm, the addition of 0.4 ppm organic Se increased the Se content in liver and serum. Suomi & Alaviuhkola (1992) concluded that Se-enriched yeast is as effective a source as inorganic Se to meet the Se requirement of pigs.

CONCLUSION

In order to prevent Se-deficiency in pigs it is necessary in the Scandinavian countries in some way to enrich pig diets. Many studies have proved that supplementation of Se to the diets is a valid method. Furthermore, these studies have revealed that pigs of different age and physiological stage do not have the same need for Se. Measured as ppm the dietare Se requirement of pigs is higher than that of older growing pigs and sows. The Se requirement in young piglets is probably met at a dietary level of about 0.40 ppm and in grower pigs at a dietary level of about 0.2 ppm, depending on the feeding strategy. Sows' requirement was not fulfilled at a intrinsic dietary level of 0.13 ppm Se, because supplementation of Se, making up a total Se content of 0.24 ppm, resulted in better performance and Se status. Thus, the dietary need for Se in sows probably lies at about 0.2 ppm. However, there are some indications that maximizing the immuno competence demands a higher dietary Se supplementation, especially in young piglets, but further studies are needed to elucidate this. If the Se supplementation to pig diets is higher than about 5 to 6 ppm, this will cause progressive harmful effects. Performance characteristics such as feed intake and daily gain are the first traits to be affected, and at higher dosages the classical toxic symptoms such as lesions in the central nervous system will occur. Ultimately, this will be lethal, but several studies have shown that pigs can survive at a quite high dietary Se supplementation, even at more than 20 ppm Se.

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21 Selenium supplementation in ruminants

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Animal production based on local feeds in Norway will always be exposed to the risk of marginal or deficient selenium intake. Adequate measures should therefore be taken to ensure that animals receive an optimal supplementation of both selenium and vitamin E.

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Myositis in lambs and calves, resembling what we now regard as selenium/vitamin E deficiency, was first described in Norway in 1934 (Slagsvold & Lund-Larsen 1934). During the late 1950s treatment with selenium and vitamin E was increasingly used to alleviate this condition and in 1961 the first commercial preparation of selenium and vitamin E as a feed additive was registered as "Tokosel®".

The first analyses of selenium from forages in Norway were made as early as in 1966 in hay from Røros, an inland area with a long history of nutritional muscular dystrophy in lambs (Mikkelsen & Aas-Hansen 1967). The levels were extremely low, ranging from 0.004 to 0.030 mg Se/kg, verifying the aetiology of the disease. Frøslie et al. (1979, 1980) presented a survey of selenium content in grains and roughage from all parts of Norway and the average results are presented in Fig. 1. Levels as well as distribution have been shown to be in agreement with later analyses of selenium content of soil made by Wu & Låg (1988).

According to Wu & Låg (1988), Norwegian soil is considered to be low or very low in selenium, with the exception of that along most of the western coast. The low selenium areas comprise the main regions of grain production, which leads to the overall low/deficient selenium content in grain in this country (Fig. 1).

In 1980 in Norway selenium was added to commercial concentrates at the rate of 0.15 mg/kg and 10 mg/kg in mineral mixtures. In 1985 these levels were increased to 0.20 and 20 mg/kg, respectively. This had, of course, a marked effect upon the total selenium content in concentrates.

Commercial concentrates selected from 1975-77 had an average total selenium content of 0.22 mg/kg (SD:0.06), ranging from 0.05 to 0.35 mg/kg (Frøslie et al. 1979). A survey carried out in 1989 revealed an average content of 0.52 mg Se/kg, ranging from 0.22 to 0.68 mg/kg (Frøslie 1991).

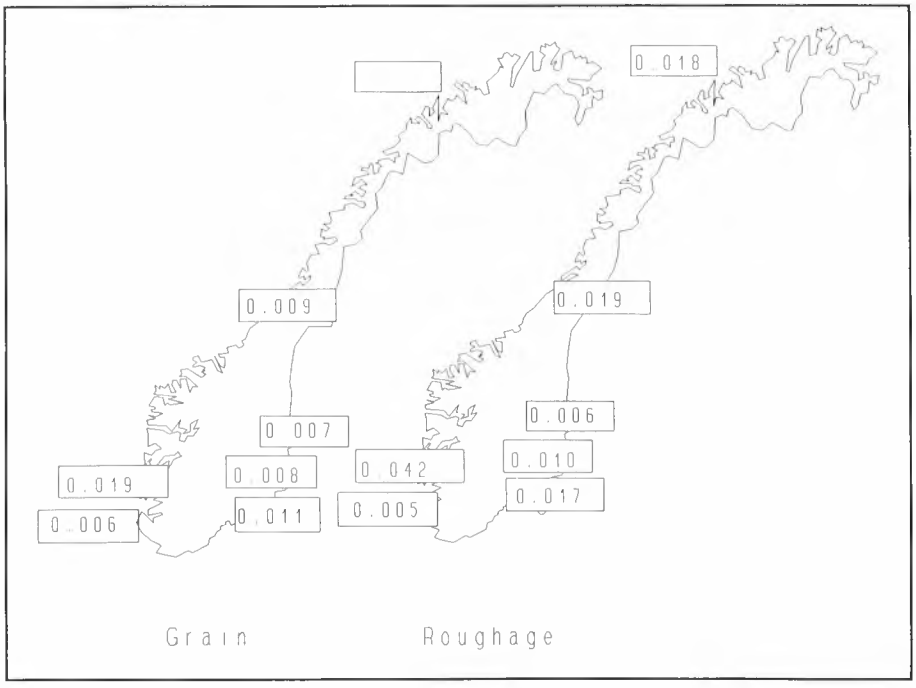

Fig. 1. Average selenium content in grain and roughage collected in different regions in Norway (from Frøslie et al. 1979)

EFFECT OF SELENIUM SUPPLEMENTATION TO FEED ON SELENIUM LEVEL IN LIVER FROM SHEEP AND CATTLE

Most of the beef in Norway is produced from the mixed beef/milk breed NRF (Norwegian Red Cattle) as a supplement to dairy production. The rest of the beef production is from the more extensive specialized beef breeds and is based upon minimal use of composed concentrates. It is worth noting the wide range of selenium values in cattle liver. There is both a geographical and seasonal variation ranging from an average of 0.14 μg/g (wet weight) in the autumn in mid-Norway to 0.30 μg Se/g during the indoor season in south-west Norway (Frøslie 1991; Øvernes & Frøslie 1992). The greatest impact upon selenium status in cattle is probably the amount of selenized concentrates in the diet. Dairy cattle and intensively fed beef cattle may have a relatively large daily selenium intake while more extensively raised beef cattle with no supplementary feed may have a daily selenium intake far below the recommended levels. Occasionally, one can observe single individuals with severe symptoms of selenium/vitamin E-dependent disorders such as muscle degeneration in such herds.

Sheep are usually sent off to slaughter immediately after return from summer pasture with no supplementary feed (except where salt licks are offered during the outdoor season)

and consequently a similar comparison with cattle would not be approriate. For sheep, however, there is a marked geographical difference in selenium content. An investigation made in 1978 revealed an average selenium content in lamb liver in eastern Norway at 0.12 μg/g (SD:0.08) and an average of 0.23 μg/g (SD:0.12) on the western coast (Frøslie et al. 1979). Autopsied lambs from south-eastern Norway in 1978 had an average of 0.15 μg/g (SD:0.10) (Frøslie et al. 1979) while autopsied lambs from the same area in 1990-91 averaged 0.31 (SD:0.12) μg Se/g (Øvernes & Frøslie 1992). This rise in selenium level is probably an effect of the selenium added to concentrates and mineral mixture.

EFFECT ON BLOOD SELENIUM IN DAIRY CATTLE

The main source of selenium in cattle nutrition is mixed concentrates. Concentrates are fed in relation to the milk yield and usually more are provided during the indoor season. Ropstad et al. (1988) followed two different herds in the course of a year and found a bell-shaped curve, with the highest blood selenium levels during midwinter ranging from 0.10 μg/ml whole blood in the autumn to 0.18 μg/ml in the spring.

In February 1992 a survey was conducted among the dairy cattle populations in Norway based on a random sampling, in order to investigate the blood selenium status (Øvernes et al. upublished results). The results showed very few geographical differences, perhaps with the exception of the surprisingly low values in the Jæren region. The material suggests that in the traditionally deficient areas, farmers are more likely to provide mineral supplements, thus levelling out the effect of the locally produced feed. However, the survey revealed that the average blood selenium in Norwegian dairy cows is at the rather low level of 0.16 μg/ml. In fact 12% of the tested individuals were below 0.10 μg/ml which is a recognized "marginal limit". Blood and Radostits (1989) even suggest that 0.15 μg Se/ml blood should be considered as marginal, in which case about half of the population is in this group. Considering that the survey was carried out in the middle of the indoor season, one should expect the average to be even lower during the summer. But then, again, vitamin E status will also be substantially higher because of the intake of fresh grass.

METHODS OF SUPPLEMENTATION

Many different methods have been used for providing ruminants with an adequate selenium supply. In Norway, up until 1980, selenium was usually administered by injection or by oral administration of high, single doses of sodium selenite or sodium hydroselenite. The toxicity risk of this regimen is relatively high (1:2 to 1:5) which was proved in practice by several incidences of often fatal selenium intoxication. Then, from 1980, by official directives in 1979, selenium has been added to compound concentrates and mineral mixtures to prevent deficiency diseases. This has reduced the incidence of selenium deficiency diseases to a minimum, and has also had an impact on animal products as described above. The same effect has been reported elsewhere (Pehrson 1984).

Ideally, a nutrient should be supplied on a daily basis through the regular feed. The method of choice will, however, vary with the management and feeding regimen. Where

a fair amount of concentrates is used, selenium should be added to the compound at the requisite level to allow for the roughage. In flocks with less intensive feeding the use of mineral mixtures or salt licks will provide a good method.

When the selenium is mixed into the feed the selenium requirement in lambs, based on the effect on selenium-dependent glutathione peroxidase activity, will be close on 0.3 mg Se/kg feed (Moksnes & Norheim 1983). In flocks given the selenium supplement through mineral mixtures or salt licks, the ewes should receive approximately 400 μg Se/day. This will result in sufficient selenium levels in blood, milk and offspring to protect against selenium deficiency diseases. This seems to be achieved with mineral mixtures and salt licks containing 20-25 mg Se/kg (Øvernes et al. 1985b).

In flocks where supplementary feed is of minor use the injection of a long-acting barium selenate preparation may be a good alternative. The dosage used, approximately 1.2 mg Se/kg body weight, is sufficient to maintain an adequate selenium status for as long as two consecutive lambing seasons (Øvernes et al. 1985a). An alternative to a depot injection is the use of ruminal pellets, either in the form of iron bullets with a grinder or as glass-boli, the latter often used in a combination of selenium, copper and cobolt (Judson et al. 1991). The efficacy and the safety of these methods seem far superior to single doses of sodium selenite.

Another way of increasing the selenium supply in ruminants is by using sodium selenate-enriched NPK fertilizer, which will result in increased selenium content in the hay (Øvernes et al. 1986).

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22 Selenium-dependent- and selenium-independent activity of glutathione peroxidase in the liver cytosolic fraction from three week-old chickens

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Both types of glutathione peroxidase (EC 1.11.1.9): selenium-dependent- (Se-GSH-Px) and selenium-independent-GSH-peroxidase (non-Se-GSH-Px) appear to be active in the removal of organic hydroperoxides at the hydrophobic membrane surface, thus inhibiting the chain reaction involved in lipid peroxidation. Non-Se-GSH-Px, which is related to the glutathione transferases or "ligandins", can be separated analytically from Se-GSH-Px since it reduces organic hydroperoxides, but not hydrogen peroxide, whereas the Se-GSHPx reduces both types of peroxides.

Butylated hydroxytoluene (BHT: 2,6-di-tert-butyl-4-methylphenol) and ethoxyquin (EQ: 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline)are antioxidants that are widely used in animal feedstuffs up to a maximum of 150 mg/kg feed. The major metabolism of both antioxidants takes place in the liver.

The purpose of the present investigation was to study the distribution of the total GSH-Px-activity in the liver cytosolic fraction from chickens on the activity of Se-GSH-Px and Non-Se-GSH-Px, and to investigate the influence of the synthetic antioxidants BHT and EQ on these enzyme activities.

MATERIALS

Twenty-four female broiler chickens (Ross) were obtained at one day old from a commercial hatchery and divided randomly into four treatment groups. A commercial diet as shown in Table 1 was used in all treatment groups and was given unsupplemented, or supplemented with BHT (150 mg/kg feed), with EQ (150 mg/kg feed) or with a combination of these two antioxidants (75 mg BHT + 75 mg EQ/kg feed). The antioxidants were included in the wheatmeal of the basal diet. The chickens received their experimental feed from the first day until slaughter at three weeks of age.

Table 1. Composition (g/kg) of basal diet

Ingredients	
Wheat[1]	250.6
Maizemeal	150.0
Peas	100.0
Rapeseed	150.0
Soyabean flour	230.0
Fat[2]	42.0
Meat and bonemeal	55.0
Molasses	5.0
Calcium carbonate	5.0
Dicalcium phosphate	5.0
Sodium chloride	1.0
Cholin chloride	2.0
DL-Methionine	0.4
Vitamin and mineral premix[3]	4.0
Antioxidant in wheatmeal[1]	2.0
Chemical analysis (g/kg DM)	
Dry matter (DM), g/kg feed	897.6
Crude protein[4]	266.3
Crude fat	146.8
Crude fibre	40.0
Crude ash	64.0
Nitrogen-free extracts	380.5
Gross energy (MJ/kg DM)[5]	15.4
Selenium	0.4

1) See text
2) The fat was a mixture of 25% soyaoil and 75% "Nowitol" (mixture fat)
3) Provided per kg of diet: 12,000 IU retinol, 3000 IU cholecalciferol, 35 mg dl-α-tocopherylacetate, 2.5 mg thiamine, 5.5 mg riboflavin, 4 mg pyridoxine, 55 mg niacin, 18 mg panthothenic acid, 1500 mcg folic acid, 200 mcg biotin, 21 mcg cyanocobalamine.
   Mineral premix provides/kg diet: 29 mg Cu, 1 mg I, 62 mg Fe, 100 mg Mn, 0.3 mg Se
4) By analysis per kg DM: 14.00 g lysine; 5.8 g methionine; 4.4 g cysteine; 9.7 g treonine. Crude protein N x 6.25
5) Metabolizable energy calculated according to NRC (1984)

METHODS

The chickens were killed by mechanical stunning and decapitation. After bleeding the livers were removed and weighed.

The livers were kept in cold 0.9% NaCl until homogenization, when 4.5 g of the liver was homogenized in 10.5 ml Saccharose-EDTA-Tris-Buffer (0.25 mM Saccharose, 1.0 mM EDTA, 5.0 mM Tris, pH=7,4). The liver cell cytosolic fraction was prepared by differental centrifugation: the homogenate was centrifuged (4°C, 10 min, 3000 x g) in an Eppendorf-5403 centrifuge. The supernatant was further centrifuged (4°C, 40 min, 19000 x g) in a Sorvall RC-5C centrifuge equipped with an SS-34 rotor and the glutathione peroxidase activity was estimated using the supernatant fraction prepared from the last centrifugation (4°C, 1 h, 50000 x g) in a Bromma LKB-2331 ultracentrifuge (70 TI rotor).

The enzyme activity was following the method by Paglia & Valentine (1967) as modified by Günzler et al. (1974), Konz (1979), and Wendel (1980). The reaction mixture consisted of 50 mM Sodium-phosphate buffer (pH 7.0), 0.5 mM EDTA, 1.0 mM NaN_3, 0.15 mM NADPH, 2 U/ml glutathione reductase, 1 mM GSH, 1.25 mM cumene hydroperoxide or 0.15 mM H_2O_2 as substrate and liver cytosol (0.2 ml) in a total volume of 2 ml. All ingredients except peroxide solution were combined and allowed to incubate for 5 min. at 37°C. Four minutes after the reaction was started by addition of substrate, the NADPH consumption rate was recorded (at 366 nm) over a period of 60 s.

Protein was assayed following the method by Lowry et al. (1951).

Calculation: The reaction with H_2O_2 was considered to measure solely Se-GSH-Px; the reaction with cumene peroxide represented the total activity of GSH-Px. The difference between these reactions constitutes the activity of non-Se-GSH-Px activity.

Data were subjected to analysis of variance using the SAS procedure. When significant F-values were obtained ($p<0.05$) treatment means were compared using the Student's t-test.

RESULTS

- Analysis of the antioxidant concentrations showed that only 121 mg BHT/kg, 85 mg EQ/kg and 67 mg BHT+29 mg EQ/kg was present in the feed.
- There was no significant difference between the different treatment groups on the Se-GSH-Px activity.
- According to Fig. 1, there was a tendency toward a higher activity of non-Se-GSH-Px in the chickens supplemented with synthetic antioxidants. The chickens fed a combination of BHT and EQ showed the highest activity of the total GSH-Px activity ($p<0.01$) and the non-Se-GSH-Px activity ($p<0.01$).
- Non-Se-GSH-Px constituted an average of 66% of total activity of GSH-Px as shown in Table 2.

Table 2. The relation (%) between the non-Se-GSH-Px and the total activity of GSH-Px activity (x ± S.D.)

Treatment Groups	Relation (%)
0 ppm	61 ± 6
150 ppm EQ	64 ± 11
150 ppm BHT	67 ± 5
75 ppm EQ+ 75 ppm BHT	72 ± 7

CONCLUSION

It is important to differentiate between the non-selenium-dependent- and the selenium-dependent activity of glutathione peroxidase in the liver, where the role of glutathione

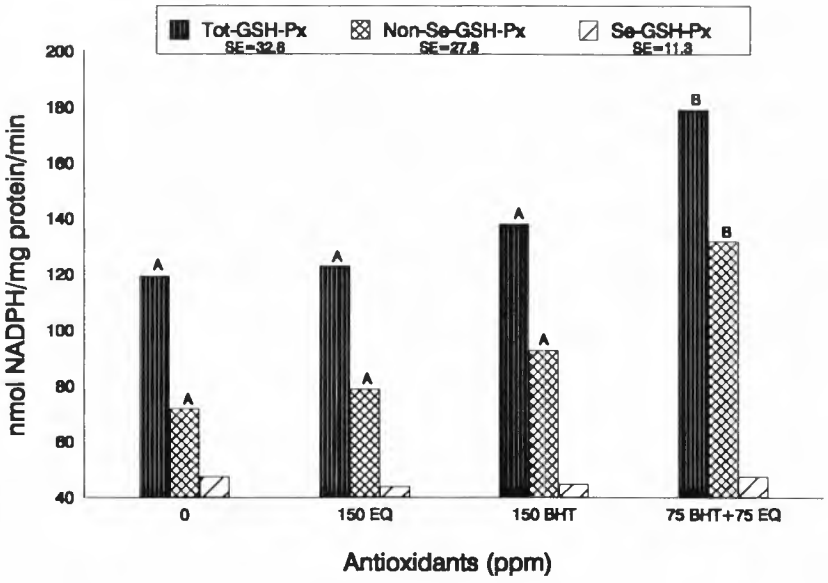

Fig. 1. Activities of glutathione peroxidase in the liver cytosolic fraction

peroxidases in hydroperoxide metabolism is concerned. The non-selenium-dependent enzyme comprised an average of 66% total glutathione peroxidase activity in liver cytosolic fraction in chickens.

The combination of the two synthetic antioxidants, BHT+EQ, seems to improve the activity of the non-selenium-dependent glutathione peroxidase, which may be important in protecting the organ against oxidative stress.

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23 Selenium content in milk during the selenium fertilization in Finland

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The selenium (Se) content in milk is linearly dependent on and readily indicates changes in dietary Se. Maximum values are reached in about 40 days after the start of supplementation, whereas the decrease is somewhat slower (Conrad & Moxon 1979; Aspila 1991). The efficiency of the transfer of Se from diet into milk is not related to either lactation stage or to age of the cow (Aspila 1991).

Se-supplemented fertilizers were first widely used in Finland in the growing 1985 season. Two levels of supplementation were used during the period 1985-90: 16 mg Se/kg (in the form of selenate) to fertilizers mainly used in cereal production and 6 mg/kg for fodder and hay production. Only the lower level (6 mg/kg) has been used since 1991.

An official monitoring programme has been carried out to evaluate the effects of Se fertilization continuously in order to get precise information on the trends in food Se. We report here on the results for fodder and milk.

MATERIALS AND METHODS

The samples of standard milk (3.9% fat) were collected every other week (from year 1992 every week). Three or four random samples (one litre each) were purchased from food stores in the Helsinki area and pooled to one analysable sample. The samples of silage (200/year), hay (100/year), oat and barley (100 of each/year) were collected from individual farms.

The samples were analysed by electrothermal atomic absorption spectrometry in the Department of Applied Chemistry and Microbiology (up until 1991 the samples of silage and hay were analyzed by hydride generation atomic absorption spectrometry at Viljavuuspalvelu).

RESULTS AND DISCUSSION

Milk was the first foodstuff to reveal the effect of Se fertilization. The Se content in milk doubled immediately after the beginning of the outdoor feeding season for cows in June

1985. During the first years of Se fertilization the Se content in milk fluctuated with the season, being about 15% lower in the outdoor season than in winter. The seasonal variation became smaller in later years. By 1989 the fluctuation was very slight and in 1990 it was almost undetectable.

In the autumn of 1991 the Se content in milk decreased significantly because of the reduced Se level in the fertilizers (Fig 1).

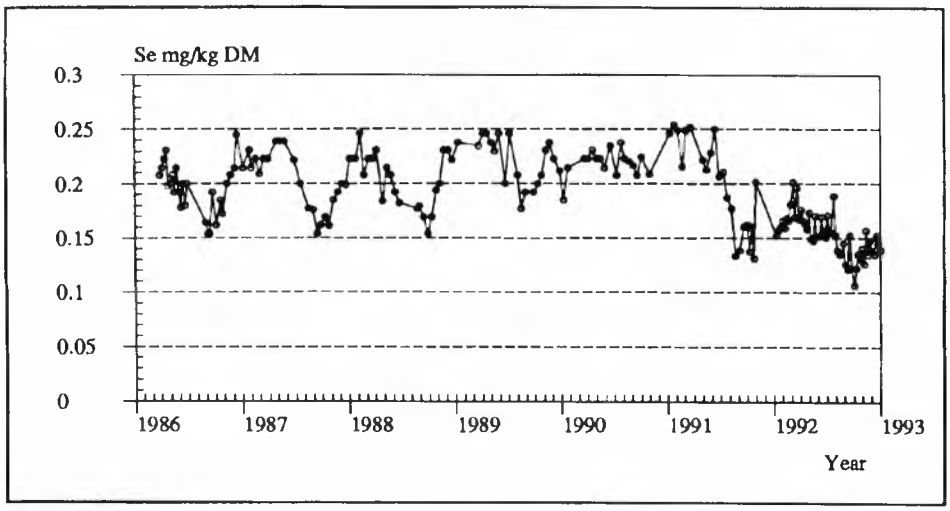

Fig. 1. Development of the Se content in milk

The Se level in milk stayed constant during the years 1987-91 despite the slight changes in the Se content in fodder (Table 1).

Table 1. The content of Se in fodder (mg/kg DM)

Sample	1986	1987	1988	1989	1990	1991
Silage	0.13	0.19	0.14	0.17	0.12	0.15
Hay	0.13	0.15	0.12	0.14	0.07	0.11
Barley	0.21	0.15	0.23	0.23	0.23	0.11
Oat	0.20	0.13	0.26	0.23	0.24	0.12
Commercial Feed	0.26	0.30	0.22	0.28	0.28*	0.28*

* the means of the years 1990 and 1991

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24 Selenium of the selenium yeast enters the cow's milk

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Finland and Estonia belong to the same European zone deficient in selenium, as do Sweden, all Baltic countries, Poland and Hungary, etc. In the countries suffering from selenium deficiency, several different ways of overcoming the risks associated with the low intake of selenium have been used. Supplementation to man has been given directly as selenite or selenate, the former even being recognized as a pro-oxidant. Both supplementation and medication of the animals due to selenium-deficiency related illnesses have been based on the use of selenite in fodder or intramuscularly, respectively.

Natural selenium yeast, grown in the presence of inorganic selenium, has been used as a source of organic selenium, where 85-90% of the selenium is found in the amino acid selenomethionine (SeMet). In addition, selenium occurs naturally in the crops mainly in the form of selenomethionine. Thus, wheat in particular can contain substantial amounts of selenomethione if grown in a soil that is able to release selenium to the grain.

A soil fertilization experiment with sodium selenate has been carried out in Finland since 1985, using 8 or 3 g selenium per hectare of cereal or grass crops, respectively. No more than a few percent of this vast amount is directed to farm animals and man - the rest is adsorbed by the soil. Even worse, the large variation in the selenium content in the crops and in the animals suggests that more advanced alternatives to selenate fertilization must be found.

Using radioactively labelled compounds, Aspila (1991) demonstrated that 0.1 mg/kg DM selenomethionine was enough to raise the selenium content of cow's milk to the 0.020 mg Se/l level, whereas 0.7 mg/kg DM of inorganic selenium was needed to produce the same effect. Pehrson et al. (1989) showed that organic selenium, pure selenomethionine and selenium from a natural selenium yeast (Selena, Leiras Pharmaceuticals, Turku, Finland) are more bioavailable for the cattle than the inorganic selenium compounds.

To show that selenium yeast can be used for supplementation to cattle in practice, we carried out a small-scale experiment in Estonia, where the soil has a low content of selenium and where the overall selenium status is also low.

EXPERIMENT

Before starting the supplementation, we took samples (muscle 0.04, heart 0.09, liver 0.07 and kidney 0.58 mg/kg wet wt.) from five slaughtered cows, one sample of pooled milk (0.010 mg/l) and samples from local hay, silage, oats, potato (all 0.013-0.014 mg/kg DM) and fodder (0.05 mg/kg DM). All values were at the same low level as that reported for Finland ten years ago (Anon. Ministry of Agriculture and Forestry. Report of the Selenium Monitoring Group. Helsinki 1990).

For the supplementation study with selenium yeast (Alkosel, Alko Ltd Biotechnology. Rajakäki, Finland), 20 dairy cows were divided into four groups of five animals and were given:

(a) yeast selenium group: 16 g Alkosel-500 ppm per day, containing 8 mg Se, i.e. 0.4 ppm in a diet of 20 kg dry wt., yeast mixed with 144 g wheat flour; (b) plain reference group; local diet only; (c) yeast reference group; 16 g inactive dried baker's yeast with flour; and (d) sodium selenite group; 0.4 ppm Se mixed flour.

Milk samples were collected every week and aliquots were pooled for the selenium assay.

After eight weeks, 4 g Alkosel-500 per day was added to the group (c) diet, which contained 2 mg Se, i.e. 0.1 ppm in a diet of 20 kg dry wt.

Selenium was analysed in all materials after digestion in the presence of H_2SO_4 and HNO_3 in a microwave digestion oven (SEM), with a hydride generation - AAS (Perkin-Elmer 200) by Dr Jorma Kumpulainen, Agricultural Research Centre, Jokioinen, Finland.

RESULTS

The selenium content of the milk from group (a) increased from the initial value of 0.011 to a high but constant level of 0.10-0.11 mg/l in three weeks. The milk selenium content in the other groups did not increase at all. From week 8, the addition of 0.1 ppm Se from selenium yeast into the group (c) diet was reflected in an increase from 0.008 to a sufficient level at 0.025 mg/l.

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