THE TRACE ELEMENTS SELENIUM,COPPER AND ZINC IN PEDIATRIC PRACTICE

THE TRACE ELEMENTS SELENIUM,COPPER AND ZINC IN PEDIATRIC PRACTICE

(DE SPOORELEMENTEN SELENIUM, KOPER EN ZINK

IN DE PEDIATRISCHE PRAKTIJK)

PROEFSCHRIFT

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PROF. DR.M. W. VAN HOF

EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.

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LIST OF ABBREVIATIONS

AE	Acrodermatitis enteropathica
CF	Cystic fibrosis
D-P	D-Penicillamine
GSH-Per	Glutathione peroxidase
Hb	Hemoglobin
P	Percentile
rpm	Rotations per minute
RDA	Recommended dietary allowances (U.S.A.)
RDI	Recommended dietary intakes (U.K.)
TPN	Total parenteral nutrition
WD	Wilson's disease

CHAPTER I. GENERAL INTRODUCTION°

1.1 THE ESSENTIAL TRACE ELEMENTS

The trace elements are so called because they constitute less than 0,01% of the weight of the human body (IUPAC ,1972).
This name is in fact quite arbitrary. It survives from the time when early investigators, with the methods then available, were experiencing great difficulties in measuring these substances in plant and animal tissues. The introduction of new analytical methods,e.g. atomic absorption spectrophotometry ,neutron activation and microwave induced emission spectroscopy have resulted in an enormous increase in our understanding of the role these elements play in metabolism.
Iron,iodine, zinc, cobalt and copper are ,since years,recognised to be essential in man : in their absence normal growth, development and/or reproduction is impossible. There is now clear evidence that selenium, chromium and manganese have also vital functions in human metabolism .They should therefore be considered essential too. Other elements possibly essential for man include molybdenum (as a component of the enzymes xanthine oxidase and sulfite oxidase), nickel, silicon ,lithium and arsenic .The evidence for the essentiality of tin and vanadium in animal nutrition is rather weak (Nielsen,1984).

° Partially published in:Clinical Nutrition 1981,R. Wesdorp et P. Soeters,(eds), Churchill Livingstone ,N.Y.,1982 .

The role and function of iron and iodide are well known and beyond the scope of this thesis.Selenium,copper and zinc will be discussed in section 1.2.

The only manifestation of chromium deficiency thus far documented in the human is an impaired glucose tolerance with fasting hyperglycemia, glucosuria, hypercholesterolemia and peripheral neuropathy which responded to chromium (Jeejeebhoy ,1977).

Several glycosyl transferases require manganese for activity. These enzymes play an important role in mucopolysaccharide metabolism (Shaw, 1980). Manganese is also required for cholesterol synthesis. Pyruvate carboxylase is among the manganese metallo-enzymes that have been isolated . Although unequivocal evidence of deficiency has not been observed in human subjects ,the existence of a deficiency syndrome has been convincingly demonstrated in animals.The features of manganese deficiency, namely impaired growth, skeletal abnormalities, depressed reproductive function and ataxia in the newborn seem to be similar in all species studied .

The function of cobalt appears to be related to its presence in the vitamin B 12 molecule. No additional cobalt is required if the intake of this vitamin is adequate (Prasad ,1978).

Fluoride functions as a structural element in osseous and dental tissues . In adequate amounts , it prevents the formation of dental cavities. There is at this moment no other known indication of a biological need (Prasad, 1978).

1.2 SHORT REVIEW OF THE ELEMENTS INVESTIGATED.

SELENIUM

In 1957,selenium was recognised to be an essential nutrient for animals .Schwarz and Foltz showed that its deficiency in rats leads to necrotic degeneration of liver, pancreas, kidney and heart.It

was not until 1979 ,that the existence of a human requirement for this element was suspected by Van Rij et al.

For a comprehensive review,the reader is referred to Schwarz (1976), Verlinden (1981), Burk (1983) and Combs (1984).

Although selenium is found in a muscle protein ,its only well characterized and essential biochemical function in mammals is in the enzyme glutathione peroxidase .It is known to be subjected to cyclic redox changes during its catalytic action .This enzyme has a major function in the removal of H_2O_2 and organic peroxides generated during oxidative metabolism in cells and tissues. Another enzyme , catalase, can also reduce H_2O_2 , but in most cells , catalase and GSH-Per are separated in different subcellular compartments . While catalase is confined almost exclusively to the peroxisomes, GSH-Per is found in the cytosol and mitochondrial matrix space .There, it provides an efficient means of defense against the build-up of peroxides which otherwise would damage cell membranes and macromolecules including DNA (Rotruck,1973 ; Flohé,1979).

GSH - Per also interacts at 2 sites of the arachidonic acid cascade, preventing the accumulation of prostaglandin G (important for the inflammatory response of tissues) and regulating prostacyclin biosynthesis .Prostacyclin inhibits the aggregation and adhesion of platelets to the endothelial lining of blood vessels (1). Platelets of selenium depleted humans show severely diminished activities of the enzyme and also altered metabolism in arachidonic acid metabolites (Kasperek,1982). Selenium plays also an important role in immune functions.In neutrophils, it is necessary for post-phagocytic cytotoxicity .Impaired candidacidal activity is an early feature in cattle deprived of this element .

Little is known about the characteristics and the clinical pre-

sentation of selenium deficiency in the human .The patients described by Van Rij have shown tenderness in the thighs and were not able to walk.This syndrome responded within a week to selenium supplementation given as sole therapy . At the same time, an endemic cardiomyopathy, occurring mainly in infants and children and known in China as Keshan disease ,was reported to be prevented with supplementation of selenium (Chen,1980). It confirmed the growing evidence that selenium might be essential for the function of the heart and other muscles in man (Editorial,1979 ; Johnson 1981; Fleming 1982) .This was the rationale for an epidemiological study done in Finland (Salonen, 1982). Reduced serum selenium concentrations proved to be correlated with acute coronary heart disease and subsequent cardiovascular death .

The requirement for selenium can be related to the degree of oxidative stress . It varies also with the supply of nutrients such as zinc, copper, manganese ,iron and the other antioxydant,vitamin E.

Little seems to be known about selenium in human fetal development. Birth of a deformed infant and miscarriages in several pregnancies amongst female laboratory technicians exposed to selenium, suggest teratogenic potential of selenium among humans (Robertson, 1970). On the other hand ,selenium prevents cadmium and arsenic induced teratogenesis . The symptoms of selenium deficiency and toxicity are species related .They are therefore difficult to interpret and extrapolate to man. Selenium has first been regarded as a toxic substance (Cooper, 1974) .This belief stems principally from the well-known selenium poisoning of cattle caused by the consumption of seleniferous plants. Paradoxically ,selenium is relatively non toxic in man (Schwarz,1976 ; Civil, 1978).According to Schwarz,clear-cut cases of selenium toxicity in the human population of the seleni-

ferous area of the United States have not been found ,in spite of careful surveys . It has been used commercially for many years with no long-term systemic effect on industrial workers. Inhaled selenium dust and fumes have given rise to irritations of the respiratory tract .Contact with selenium dioxide causes dermatitis and burns.

Organo-selenium compounds seem to have a wide range of toxicity in the laboratory animals studied.Dietary selenium apparently induces cancer in rodents.In humans, however, epidemiological evidence suggests that selenium compounds may have a therapeutic value against cancer (Schrauzer ,1977). There are at this moment no direct reports about selenium carcinogenicity in humans. As for other metals, the difference between toxicity and essentiality is not due to the nature of the element involved but to the dosis received.The daily intake for a adult of 70 Kg varies from 30 to 300 ug / day , according to the country considered.In adults, signs of toxicity (gastric problems,liver disease) occur at intake levels of approximately 700 ug / day. Astronomical intakes (up to 1 g of selenium) have been reported to cause death.Two children with cystic fibrosis died after chronic intake of high doses of selenium compounds, purchased in health stores (Hubbard,1980).

COPPER

In 1928 , Hart et al. showed copper to be an essential nutrient for the rat .They speculated upon its possible significance for human metabolism .In 1935 ,this was partially documented by Elvehjem when he showed that copper could be effective in treating anemic infants. For a comprehensive review ,the reader is referred to Shaw (1980) and to Danks (1983).

Copper is now known to be a component of a number of metalloenzymes. The major ones are caeruloplasmin, cytochrome C oxidase,superoxide

dismutase, lysyl oxidase which catalyses the cross-linking of the polypeptide chains of collagen and elastin , and finally tyrosinase which catalyses the first two steps in the biosynthesis of melanin. Copper deficiency is thought to interfere with heme synthesis in three ways: a.by diminishing iron transport into the mitochondria, b.by decreasing the activity of ferrochelatase, c.possibly by reducing the activity of the copper iron enzyme cytochrome C oxidase. Neutropenia and anemia resistant to iron therapy are regular features in copper deficient animals and infants (Lardy, 1978) .Later on bone changes reminiscent of scurvy may develop with occasional fractures. Copper is accumulated in the liver during the latest weeks of pregnancy (Widdowson,1972).Preterm infants weighing less than 1200 g are especially at risk , showing deficiencies 3 to 6 months after birth (Ashkenazi, 1973).

The full blown syndrome of severe copper deficiency is observed in a lethal X linked inherited disease first described by Menkes et al.(Danks, 1972). There are many of the features of copper deficiency (pili torti, typical bone changes, arterial intimal changes, cerebellar degeneration with hypotonia, seizures and developmental regression, hypothermia) as observed in the laboratory animal but apparently without neutropenia and anemia. Although copper absorption is altered ,the defect is not limited to transport alone, but seems to affect distribution as well (Danks,1977).

Little seems to be known about the effect of copper deficiency during human pregnancy . Copper must enter the embryo from the earliest stages of gestation.Copper deficiency during pregnancy in sheep results in the ataxic disease "swayback" in the newborn lamb. In affected animals there is an extensive demyelination of the CNS. The brain mitochondria show a depletion of cytochrome C oxidase.

These changes are also observed in a number of other species (Hurley,1976).

With the exception of prematurely born infants fed on TPN and elemental diets (Sivasubramanian,1978 ; Heller, 1978),nutritional evidence of copper deficiency is extremely rare . Its requirement seems to be quite low (Shaw,1982; Wilson, 1960).

Copper toxicity is well recognized . It occurs rather frequently as a consequence of massive contamination or as a consequence of Wilson's disease .

Copper poisoning,for example,is seen in Indian Childhood Cirrhosis. Brass and copper household utensils are the most probable source of the gross accumulation of copper in the liver (Tanner,1979).

Wilson's disease (Wilson,1912) is a autosomically recessive inherited metabolic disease (prevalence 1 in I00.000 live births) .It is characterised by a progressive cirrhosis of the liver, proximal renal tubular malfunction with glucosuria and aminoaciduria, hepatolenticular degeneration,osteoporosis and arthropathy and Kayser-Fleischer rings in the cornea . This is due to the pathological accumulation of copper in the tissues. In Wilson's disease ,both biliary excretion of copper in the bile (Danks,1983) and incorporation in caeruloplasmin are severely impaired (Vierling,1978). The basic defect underlying this entity is not yet known.If not adequately treated ,the disease is fatal.

ZINC

In 1869, Raulin showed that zinc is essential for the growth of the fungus Aspergillus Niger. It was not until 1934 that Todd et al. demonstrated that it was an essential nutrient for a mammalian species - the rat .Not until 1963 had Prasad documented the

occurrence of zinc deficiency in the human and thus established the essentiality of this metal for man.In 1973,Barnes and Moynahan recognized the fundamental role of zinc in the pathogenesis of a rare inherited disorder :acrodermatitis enteropathica (AE).

For a comprehensive review,the reader is referred to Aggett(1979) Gordon (1981),Hambidge (1981),Prasad (1983 ,1984).

The first concrete demonstration of a specific biological function, critically dependent on the presence of zinc ,came in 1940. Keilin and Mann found that carbonic anhydrase contained zinc that was essential to its mechanism of action.If related enzymes from different species are included, more than 200 zinc proteins, most of them enzymes, are presently on record (Vallee,1984).

Zinc is present in the enzymes in a fixed stoichiometric amount and its presence is essential to the catalytic function of the enzymes: removal of the zinc with chelating agents often leaves an inactive apoenzyme but full activity can generally be restored by the addition of zinc. Three enzymes,alkaline phosphatase,carboxypeptidase and thymidine kinase appear to be most sensitive to zinc restriction. It is also established that the DNA - and RNA polymerases and reverse transcriptases of numerous prokaryotic and eukaryotic organisms are zinc enzymes (Lieberman,1963). This explains the essential role of zinc in the replication and transcription of DNA during cell division.

Aside of their function as an integral part of a biologically important protein, the zinc ions seem to participate in the regulation of enzyme activity. Zinc ions,for example, have a specific role in modulating cell membrane function probably by antagonizing calcium at the calmodulin binding sites (Brewer,1980).Recent evidence indicates that zinc plays a crucial role in immune functions (Chan-

dra,1980): not only does it exert a profound and apparently specific effect on the thymus and thymocytes (Good,1979) but also on granulocytes (Briggs,1982).Abnormal granulocyte chemotaxis,corrected by zinc supplementation has indeed been observed in patients with zinc deficiency.

Growth retardation, poor appetite, mental lethargy, skin changes and hypogonadism with delay in sexual maturation in the male were the typical clinical features of chronically zinc deficient subjects in the Middle East (Prasad in 1963). These features were corrected by zinc supplementation alone.

Recently abnormal dark adaptation has also been related to a deficiency of zinc(Morrison,1978). This effect is probably mediated by the enzyme retinol dehydrogenase which is present in the retina and known to be zinc dependent.

In 1974,Moynahan reported the features of profound zinc deficiency in patients with acrodermatitis enteropathica .This is an inherited disorder which can be treated by giving pharmacological doses of zinc. This disorder includes alopecia and vesicopustular lesions of the face, perineum and extremities.These are combined with paronychia ,esophagitis, diarrhea, a pronounced thymic atrophy and a high incidence of candida albicans infection .Left untreated this condition becomes fatal .

The role of zinc in testicular function was investigated in rats (Ley,1976).These data indicated a specific effect on testes and suggested that testicular function in the zinc deficient state is affected through some alterations of testosterone biosynthesis in the male.

A very high incidence of low birth weight and congenital malformations in children of patients with partially treated AE was reported

by Hambidge et al. (1975).This suggests that zinc deficiency results in intrauterine growth retardation and congenital malformations in man as it does in animals.The results of zinc deficiency during pregnancy in the rat have indeed been described by Hurley and her colleagues (1973, 1976). Severe zinc deficiency resulted in total infertility. Less severe deficiency reduced conception by 50%, and the young animals weighed less than 50% of the control. Gross congenital malformations were present in 98%: cleft palate being the commonest followed by anophtalmia, anencephaly, hydrocephaly and fused or missing digits. According to Jameson (1976) zinc deficiency during pregnancy in the human female is characterised by increased maternal morbidity and increased risks (especially postmaturity)to the fetus. A possible correlation between a low maternal serum zinc level and congenital malformations mostly of the central nervous system has been postulated.The relation between maternal zinc deficiency and the fetal alcohol syndrome needs further investigation.
The human requirement for zinc seems to be higher than anticipated (Halsted,1974 ; Zlotkin, 1983). It has now become obvious that nutritional deficiency of zinc in the human is fairly prevalent throughout the world even in developed countries (Sandstead,1973) and in well-nourished children . Hambidge et al.(1972; 1976) identified ten children aged 4 to 13 years from middle and upper income class families.They had retarded growth and significantly decreased zinc levels in the hair .Old age, pregnancy and lactation have been associated with higher incidence of poor zinc status,as well as alcoholic liver cirrhosis and several other diseases associated with poor nutrition (Halsted,1970). Since the first observation of Kay and Tasman-Jones in 1975, numerous reports of a syndrome ressembling AE occurring in patients of all ages receiving zinc-free

TPN leave little doubt that zinc should be included in all TPN solutions (ref.25,1979 ; Latimer,1980).

Compared to other metals as lead or arsenic, zinc is not very toxic. Pharmacological doses of zinc have been used without significant signs of toxicity.Oral ingestion of 12 gram of zinc sulfate taken over a two-day period caused slight drowsiness,lethargy and an increase in serum amylase and lipase levels(Murphy,1980).

Inhalation of metal fumes has been known for many years to cause a clinical picture similar to an upper respiratory infection and at times pneumonia (Papp, 1968).

1.3 OUTLINE OF THE NUTRITIONAL ASPECTS OF TRACE ELEMENT METABOLISM

DEFINITIONS

Since growth and development require not only energy but also an adequate supply of all necessary nutrients,proper nutrition is of utmost importance during infancy and childhood .It is thus mandatory to provide essential trace elements according to the needs.

The problem relies in estimating and specifying these needs.This is indeed of great practical importance for preventing ,detecting and treating deficiencies or abuses.Therefore it is necessary to establish the requirement of an individual for a specific nutrient. The requirement is a physiological concept .It may be defined as the minimal amount of that nutrient that will promote and maintain an optimal state of health (Fomon, 1974) .This is not an absolute value independent of other variables.Ideally the advisable intake of a nutrient should be identical to the requirement.But estimates of requirements include a considerable element of uncertainty .Therefore advisable intakes are set at values greater than the estimated

requirements.Although all recommendations must ultimately be based on data concerning requirements ,it should be noted that both recommended dietary allowances(RDA) and recommended dietary intakes (RDI) are not identical with requirements.

The U.S.National Academy of Sciences(Committee on Dietary Allowances of the Food and Nutrition Board of the National Research Council) defines the recommended dietary allowances as: the levels of intake of essential nutrients considered,on the basis of available scientific knowledge,to be adequate to meet the known nutritional needs of practically all healthy persons (51).

The U.K. Department of Health and Social Security defines the recommended dietary intakes for nutrients as the amounts sufficient or more than sufficient for the nutritional needs of practically all healthy persons in a population (77). Both RDA and RDI serve to evaluate and program the food supply of a population group. They are usually "high" enough to cover substantially all individual variations in the requirements of normal people .They include a margin of safety above the critical level of each nutrient.Therefore, the FAO/WHO introduced in 1973 the term safe level of intake: "the amount of protein considered necessary to meet the physiological needs and maintain the health of nearly all persons in a specified group" (59).This concept, defined here for protein, can be extrapolated to other nutrients.Instead of the usual recommended dietary allowance ,"safe and adequate intakes" are recommended for micronutrients that are clearly essential when a large degree of uncertainty exists concerning the requirement (Rosenberg, 1985). This concept also has the advantage of introducing the idea of risks and therefore of probability.A person who has an intake lower than the safe level is not necessarily deficient but the lower the intake,

the greater the probability to become deficient.

METHODS OF ESTIMATING REQUIREMENTS

Different methods have been devised, especially in infants, to estimate the order of magnitude of the individual requirement.

For a comprehensive review the reader is referred to Fomon (1974, 1979), Committee on Nutrition (1979), Rosenberg (1985).

The mineral content of human milk has been used preferentially for the calculation of trace element requirements for infants.Iron and zinc from human milk have been found to be uniquely and very efficiently absorbed by the infant. Consequently formulas and elemental diets had to be supplemented with higher amounts than expected in order to meet the requirement.

Theoretically based calculations have also been used extensively to determine the requirement for infants .For example ,the requirement for zinc may be calculated from the estimated increase in fat-free tissue and on the assumed value for zinc concentration in this newly formed tissue (1,9 mg zinc/100 g lean body mass in the newborn). Between birth and the age of 4 months an infant has to retain 320 ug of zinc daily to assure a normal increment in lean body mass.

Many published allowances ,suggested by various investigators, are based on balance studies and clinical experience with administration of trace elements in TPN solutions .They are surprisingly close to each other and are actually extensively used in clinical settings. Reports of intakes by individuals who developed symptoms of a deficiency (indicating inadequate intakes) and reports of intakes by healthy individuals allow to calculate the dietary requirement with a large security margin.

Aside from direct clinical experience ,many investigators have also

extrapolated data from human adults to infants or children , and from the young or adult animals to human infants.

Table.Safe and adequate levels of intakes of essential trace elements for infants (Tentative estimates)°

	Oral (mg /day)	Intravenous (ug/Kg/day)
Zinc	3 - 5 mg	100-500 ug
Copper	0.5 - 0.7 mg	20 ug
Chromium	0.01- 0.04 mg	0,1-0,2 ug
Manganese	0.5 - 0.7 mg	2 - 5 ug
Selenium	0.01- 0.04 mg	0,5-2 ug

°Fomon (1979), Committee on Nutrition (1976), Ricour (1977), Zlotkin (1983),Hambidge (1983)

FACTORS INFLUENCING REQUIREMENTS

This Table reflects only provisional and tentative estimates of intakes .Many different factors are known to affect the requirement of a particular essential trace element :

The composition of the diet may influence the requirement extensively. For example a high-energy diet without an adequate mineral intake enhances a deficiency state.

Insufficient absorption may increase the requirement of the element. Because of the presence of phytate ,fiber and other complexing substances in soy and cereal protein , the requirement of zinc in a vegetarian diet may be higher than in a mixed type diet. Zinc may also be insufficiently assimilated in the presence of steatorrhea and/or diarrhea.

Increased losses may occur either via the gastrointestinal tract or via other routes. It may be excreted via the urine in presence of proteinuria and aminoaciduria ,or through the skin with increased loss of sweat or following wounds and large surface burns.Alcoholism

induces also an important loss of zinc via the urine by an unknown mechanism.

Trace elements should be given in a biologically active form , available for metabolic processes.For example,it is questionable whether the chromium present in intravenous solutions as contaminant is utilised.The bioavailability may greatly be influenced by the presence of other elements in the diet : the absorption of copper is reduced in the presence of large zinc intakes as the availability of zinc is also reduced in the presence of iron.

The requirement varies according to age and growth rate. The premature infants have smaller stores of energy and nutrients than the full term ones.They are physiologically less prepared to maintain homeostasis . They have to gain a more rapid lean body mass in the first months of life in order to resume intra-uterine growth rate. Their intakes will consequently have to be adapted accordingly .

It is also to be remembered that the requirements of severely ill patients are higher than those of healthy individuals.

1.4.PURPOSE OF THIS STUDY

To allow normal growth and development there are good reasons for giving iron,iodide,zinc,cobalt (as vitamine B 12),copper, and less known essential trace elements such as selenium ,manganese,chromium, and possibly fluoride and molybdenum.

The purpose of this study was to investigate and describe some aspects of the trace element metabolism in infancy and childhood. In chapter II, reference values of plasma selenium concentrations in Belgium and The Netherlands are given . The influence of age is discussed. Annotations on serum zinc and plasma copper are added for reason of completeness. There are no doubts that the nutritional intake which is geographically related ,exerts a profound influence

on the mineral status of a given individual.

Therefore ,in chapter III we focused on the different aspects of the selenium intake of infants ,both in foods and in parenteral solutions. Reports of trace element deficiencies in patients receiving parenteral nutrition and/or synthetic diets emphasized the need for more information on requirements.

If the child is unable to absorb these elements in sufficient amounts , they should either be supplemented with the foods, or provided parenterally. With this purpose , we studied in chapter IV the selenium ,copper and zinc status in 13 patients with cystic fibrosis.

Four children with Wilson's disease were investigated. The problems were multiple. The accumulation of copper in the liver and other tissues as the effects of the copper chelating therapy had both to be taken into consideration.In chapter V.1, the effect of D-Penicillamine therapy is described .In Chapter V.2 the effect of zinc-copper interaction during zinc sulfate therapy is discussed.

In summary,examples of 4 different pathological mechanisms and their effect upon the metabolism of selenium ,copper and zinc will be presented :1) inadequate intake as seen in TPN 2) malabsorption represented by cystic fibrosis 3) increased urinary losses caused by the chelating agent D-Penicillamine 4) competitive inhibition of copper absorption through pharmacological doses of zinc sulfate.

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CHAPTER II.REFERENCE VALUES

2.1 INFLUENCE OF AGE ON THE SELENIUM STATUS IN BELGIUM AND THE NETHERLANDS.

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SUMMARY

Plasma selenium concentration and glutathione peroxidase activity (GSH-Per) in red blood cells were determined in subjects from different age groups. The selenium level (mean ± SD) found in infancy (0 to 6 months) was 2 ± 0.6 ug/100 ml, with the lowest value of 1 ug/100 ml observed in a 4 months old infant. These levels were significantly lower ($p<0.001$) than the value of 9.5 ug/100 ml ± 1.1 found in the adult group and 7.7 ug/100 ml ± 1.3 found in the group of older children (2 to 15 years). Younger children (6 to 24 months) had intermediate levels of 5 ug/100 ml ± 1.2. When the data were plotted on a logarithmic scale as a function of age the figure shows clearly that the plasma selenium levels increase steadily with age throughout life after an initial drop at 60 to 90 days.

There was a satisfactory correlation between the plasma selenium concentrations and the GSH-Per activity in the red blood cells ($r=0.45, p<0.005$). Although very low selenium values were observed the GSH-Per activity remained (with only one exception) in all patients > 10 U/g Hb.

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INTRODUCTION

Selenium is an essential nutrient for animals: it was recognized as such in 1957 when Schwarz and Foltz showed in rats that its deficiency leads to necrotic degeneration of liver, pancreas, kidney and heart (12). It was not until 1979 ,however,that the existence of a human requirement for this element was described, when van Rij et al. observed a patient on total parenteral nutrition who developed tenderness in the thighs with inability to walk. This syndrome responded within a week to selenium supplementation given as sole therapy (17). Almost simultaneously an endemic cardiomyopathy, occurring mainly in infants and children and known in China as Keshan disease, was reported to be prevented with supplementation of selenium (2), confirming the growing evidence that selenium might be essential for the function of the heart and other muscles in man. Although selenium is found in a muscle protein (12), its only well characterized and essential biochemical function in mammals is in the enzyme glutathione peroxidase (GSH-Per, EC 1.11.1.9), which is known to be subjected to cyclic redox changes during its catalytic action (3). This enzyme is thought to have a major function in the removal of H_2O_2 and organic peroxides generated during oxidative metabolism in cells and tissues, providing an efficient means of defense against the build-up of such peroxides which otherwise are able to damage cell membranes and macromolecules including DNA (13). It also interacts at 2 sites of the arachidonic acid cascade, preventing the accumulation of prostaglandin G (which might be essential for the inflammatory responses of tissues) and regulating prostacyclin biosynthesis which inhibits the aggregation and adhesion of platelets to the endothelial lining of blood vessels (1). Selenium levels in adult man have been shown to correlate with the

selenium content of soils and foods (15). The determination of the selenium requirement is therefore of particular importance in those countries where the selenium intake is on the borderline, as is the case in Belgium and The Netherlands (18). Few data are presented in the literature on the selenium status of infants, children, adults and elderly in Western-Europe. To obtain this information plasma selenium levels and activity of GSH-Per in red blood cells were determined as possible functional parameters of adequate selenium status in subjects from different age groups.

MATERIALS AND METHODS

Blood samples were obtained from 25 infants (0-24 months), all receiving the same humanized cow's milk infant formula (Almiron® - Nutricia) and from 16 children (2-15 years) who had to undergo either minor operations of diagnostic procedures or were seen at the outpatient department at a last follow up visit after an acute illness. All were growing well and had a height and weight > P3 for age. Ten adults were members of the laboratory staff, 5 were healthy relatives and 5 were seen at the Department of Medicine for minor complaints.

All samples were collected and stored in metal free tubes. After centrifugation at 2800 rpm, plasma and red blood cells were separated and kept frozen at -70° until analysis. The glutathione peroxidase activity of the red cells was measured at 30° in a Zeiss PMQ III spectrophotometer, using the coupled test procedure with the substrate t-butylhydroperoxide as described by Paglia and Valentine (8) and was expressed as units per gram hemoglobin . One unit was defined as the amount of enzyme that would catalyse the oxidation of 1 nM.NADPH/min. under the conditions of the assay. Storage of frozen

samples at -70° was shown to have no effect on enzyme activity. Reduced glutathione, GSSH reductase and NADPH were obtained from Boehringer Mannheim.

The plasma selenium concentrations were determined by the fluorometric method of Watkinson (19). 2.3-Diamino-naphtalene (Fluka) was used as complexing reagent and cyclohexane (Baker) as the extracting solvent for the complex. The fluorescence was measured in a Baird Atomic fluorometer, model SF-1. Excitation wave length = 366 nm, fluorescence wave length = 516 nm. The selenium content was expressed as ug/100 ml.

RESULTS

Figure 1 reports the plasma levels of selenium from all 61 subjects as a function of age on a logarithmic scale. The solid line is a computer-drawn cubic spline fitted on the data points. As can be seen from the figure, the mean (± SD) plasma selenium concentrations found in the very young infant group (0 to 6 months) reaches the low values of 2 ± 0.6 ug/100 ml (range: 1 -3.2 ug/100 ml), the 5 infants younger than 60 days showing a level slightly higher (2.2 ± 0.5 ug/100 ml) than those 60 days and older (1.8 ± 0.8 ug/100 ml). The lowest value (1 ug/100 ml) was found in a 4 months old infant. These levels were significantly lower ($p < 0.001$) than the value of 9.5 ± 1.1 ug/100 ml found in the adults (range: 7.2 - 11.6 ug/100 ml) or the value of 7.7 ± 1.3 ug/100 ml found in the older children (range: 5.3 ug/100 ml - 10.4 ug/100 ml).

Younger children (6 to 24 months) had intermediate levels with a mean (± SD) of 5 ± 1.2 ug/100 ml (range: 2.8 ug/100 ml - 7.3 ug/100 ml). The figure shows also that the plasma selenium value increases steadily with age throughout life, after an initial drop at about 4 months of age.

Figure 2 shows the correlation between the plasma selenium concentration and the RBC glutathione peroxidase activities in 44 subjects ($r=0.45$; $p < 0.005$). Although very low selenium values were observed, particularly in the very young infants, the glutathione peroxidase activity remained at all time but once above 10 U/g Hb. Most remarkably the mean activity found in the group of young infants (18.2 $\pm$ 6.8 U/g Hb) did not differ significantly from the value found in the 6 to 24 months old group (18.1 $\pm$ 6.8 U/g Hb) nor from the value found in the group of older children (22.5 $\pm$ 7 U/g Hb). Adults reached the highest levels of activities (27 $\pm$ 8 U/g Hb); range: 10.5 - 40 U/g Hb), although those, taken as a group, were not statistically significantly different from the levels found in infancy.

DISCUSSION

Only a few studies on the selenium status of growing children have been published (5,7,20). The plasma selenium concentrations we report here in infants fed humanized cow's milk formula and in children and adolescents with an adequate nutritional status as assessed by growth parameters agree well with the findings of Lombeck in Germany (5). It confirms the fact that selenium in man as well in the animal (9) is age dependent, which would support the existence of a maturation process. It also extends this information to the Benelux countries (Belgium - The Netherlands - Luxemburg) of Western-Europe.

The lowest values were observed in infants 60 to 90 days old, and reached levels (1 ug/100 ml) comparable to those reported in adults with clinical selenium deficiency (17). The plasma selenium levels of healthy adults from The Netherlands are comparable to the values

found in Germany (5) and in Finland (20). They are at least 2 to 3 times lower than the levels observed in the residents of the United States, Canada or Japan where values up to 17-22 ug/100 ml are found (12). According to Valentine a deficient status is improbable with plasma selenium concentrations above 10 ug/100 ml (16). As can be seen from the figure the levels reported here in the adults are borderline and those observed in infants and children dramatically low.

Selenium levels have been reported to correlate well with the selenium content of the diet (15). Calculated daily intakes are low in The Netherlands but also in all the North of Western-Europe including Sweden, Finland and West-Germany (18). The extremely low levels seen in infancy (circa 10% of adults values) are most probably due to the low selenium content of cow's milk as compared to human milk (6), but could also be due to the insufficient availability and/or storage of selenium during pregnancy, the cord levels being a function of mother's selenium status (10). This latter, as seen on the figure, is on the borderline and sometimes even inadequate in the countries where the daily intake of selenium is low.

As others (3,5,7,13), we found a positive relationship between plasma selenium levels and the GSH-Per activity in the red blood cells. The fact that this relationship exists suggests that the selenium requirement is not met: indeed the enzyme activity, in adults, depends strongly on the selenium supply until a plateau of activity is reached (3). There is now accumulating evidence that the activity of GSH-Per is submaximal at selenium concentrations of less than 10 ug/100 ml. Noteworthy in this respect are the GSH-Per activities found in the group of young infants. The activities are lower but not significantly different from those seen in older

children and adults, despite the very low selenium values. The question has to be raised whether this represents a residual activity reflecting Se status at the moment of red blood cell formation or the activity of a non-selenium dependent GSH-Per in this age group. The existence in Western Europe of a causal relationship between low selenium levels and a clinical disorder has not yet been convincingly demonstrated. Nevertheless a subacute deficiency existing for some time might induce a metabolic or histopathological change which on its turn could trigger a disease at some future time when the deficiency itself may not be detectable anymore (3). In this prospect, the recently reported study describing the correlation between a subacute selenium deficiency and myocardial infarction and cardiovascular diseases is particularly important (11). Further epidemiologic studies are urgently needed. A more practical and direct way to detect and quantify lipid peroxidation and the influence of the selenium status on this process is probably the measurement of alkanes like ethane and pentane (4).

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Fig. I

Selenium concentrations in plasma of 61 individuals of different ages.

Conversion factor ug/100 ml to umol/l : 0.126 .

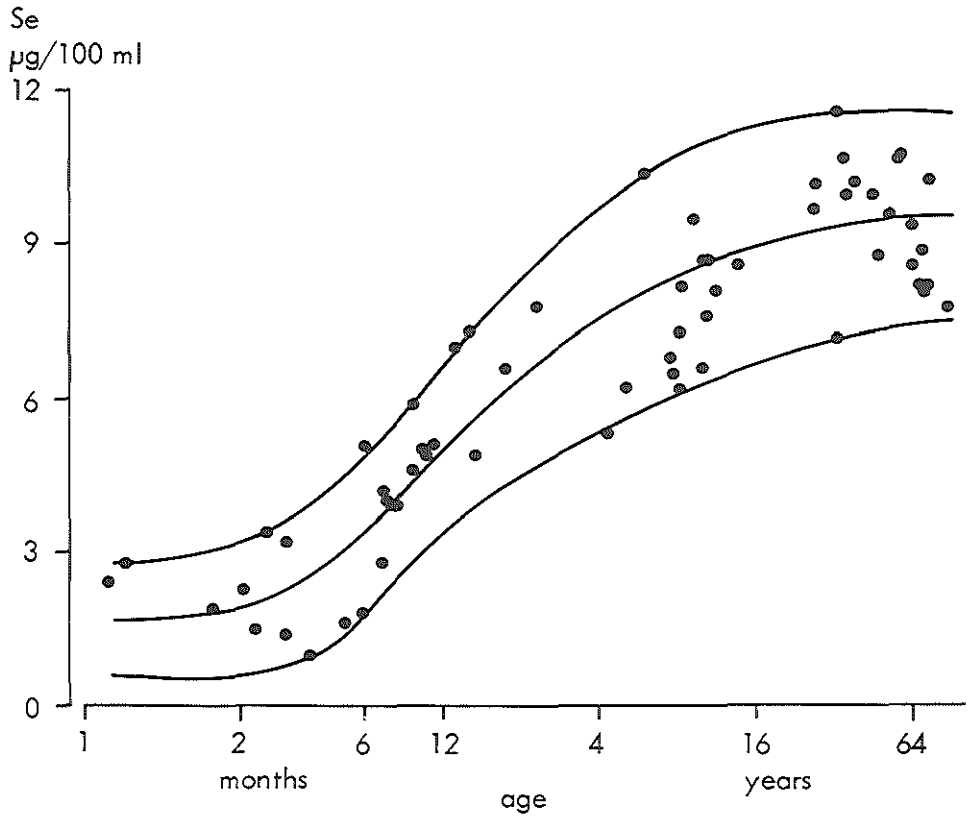
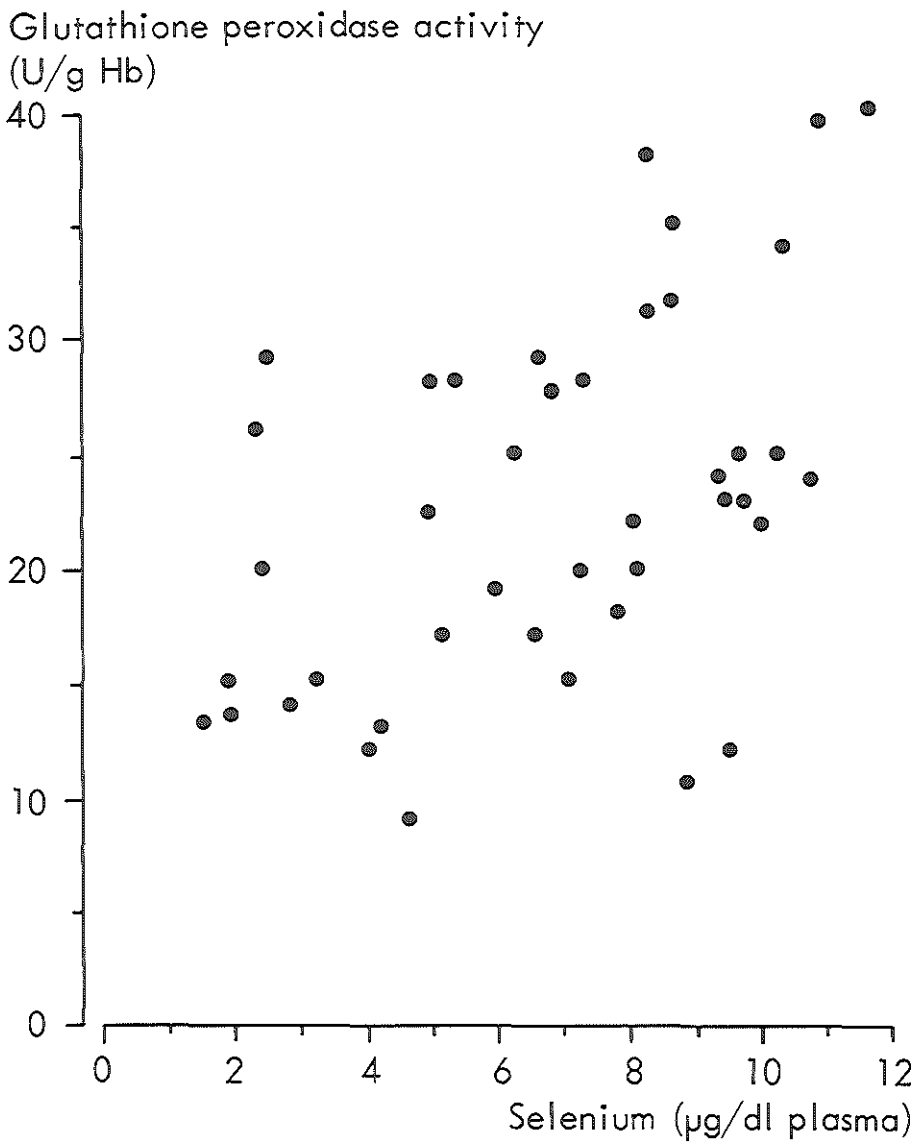


Fig. II

Plasma selenium concentrations and glutathione peroxidase activities in red blood cells of 44 individuals of different ages.



2.2 ANNOTATIONS ON PLASMA COPPER AND SERUM ZINC CONCENTRATIONS

PLASMA COPPER CONCENTRATION

Copper is an essential trace metal,involved in cell and tissue growth.Dietary deficiency of copper is extremely rare.It is seen almost exclusively in very low birth weight premature infants (1),and in term infants on total parenteral alimentation without copper supplementation (2).Menkes kinky hair syndrome has been recognised by Danks et al.in 1972 as a congenital and inherited disease of copper metabolism (3). Most problems are due to accumulation of copper into the tissues (4,5).

We assessed in our laboratory the plasma copper values in a group of 14 children older than 2 years , who had to undergo either minor operations or diagnostic procedures or were seen at the outpatient department at a last follow-up visit after an acute illness.

All samples were collected and stored in metal free containers . After centrifugation at 2800 rpm ,the plasma was kept at -70°,until analysis.The copper content was estimated in duplicate by atomic absorption spectrophotometry ,using a graphite tube (Perkin - Elmer 303) .We compared the data obtained with the scarce but classic pediatric reference values reported in the literature ,which are summarized in the Table.

The results obtained (120 $\pm$ 22 ug/100 ml, range: 92 - 151 ug/100 ml) are comparable to those previously described .

Plasma copper concentrations (6, 7).

Age	Number	Mean Values (ug /100 ml)	SD (ug/100 ml)	Range (ug/100 ml)
Birth	15	29	3	20 to 70
2 to 6 months	8	64	5	
6 month to 5 years				27- 153
5 to 17 years				94- 230
Adults	82	106	18	70- 118

Conversion factor ug/100 ml to umol/l : 0.157.

As shown by Henkin (6) and Scheinberg et al.(8),the mean concentration of total plasma copper at birth was significantly lower than the adult level .It showed a consistent rise during the first months of life, until the age of 8 months .At this age it remained relatively stable for the next 13 months at values significantly above the adult levels (6).

Mean nondiffusible copper and ceruloplasmin levels in the newborn infant are about a fourth of the adult values. They show a subsequent gradual increase , which closely parallel the change observed in total plasma copper concentration. In the later period of infancy,and up to 6 years of age the copper level is higher than in the adult .From the age of 6 years till the age of 13 to 16 years, the total plasma copper concentration decreases gradually to the adult value .

These data showed a specific profile of plasma copper concentration during infancy,different from that of plasma zinc.It is therefore remarkable that while serum levels of copper and ceruloplasmin are

the lowest at birth, the copper content of fetal and neonatal tissues, particularly liver, muscle, skin, adrenal glands,thyroid, testes,and uterus is much higher than in adults (9). These tissue levels decrease soon after birth while there is a corresponding rise in plasma copper and ceruloplasmin concentrations (10).

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ZINC

Zinc has been known to be an essential trace metal for mammals since 1934 (1) and for man since 1963 (2) .The hypothesis that the nutritional zinc intake in the western world might at times be marginal, could have been seen as ridiculous until several years ago. Deficiencies were first documented in 1973 (4) and later confirmed by Walravens and Hambidge in a group children from low - and middle class American families in Denver (5).

Quite rapidly since those first reports it became clear that human zinc deficiency may occur under certain circumstances, even in the presence of an adequate energy and protein intake.

A severe acute zinc deficiency has been described in patients with acrodermatitis enteropathica (6)and in patients treated with long-term parenteral alimentation without zinc supplementation (7).

Marginal, mainly secondary zinc deficiency has since been observed in patients with the syndrome of protracted diarrhea in infancy (8), with coeliac disease (9) ,in very low birth weight infants fed parenterally (10), in adults with cirrhosis (11), with Crohn 's disease (12) and in a continuously growing number of other pathological entities (13).

We assessed in our laboratory the values of serum zinc concentrations in healthy individuals of different ages . Serum samples were obtained from 21 infants (age: 0 to 24 months), all receiving the same humanised cow's milk formula (Almiron R -

Nutricia) and from 18 children (2 to 15 years) who had to undergo either minor operations or diagnostic procedures or were seen at the outpatient department at a last follow-up visit after an acute illness.All were growing well and had a height and weight > P 3 for age.The samples were drawn between 8 and 12 a.m.

In 11 healthy adults the zinc levels were determined ,and in 7 this was done in fasting and non fasting condition.

All samples were collected and stored in metal free containers . After centrifugation at 2800 rpm ,the serum was kept at -70°,until analysis.The zinc content was estimated in duplicate by atomic absorption spectrophotometry, flame detection, Perkin- Elmer 303.

We compared the data obtained (Table below) with the pediatric reference values reported in the literature.

Serum zinc concentrations.

Age	Number	Mean Values (ug /100 ml)	SD (ug/100 ml)	Range (ug/100 ml)
2 to 5 months	9	92	23	61 - 136
6 to 24 months	12	103	14	85 - 124
2 to 18 years	18	101	10	90 -120
Adults	11	117	20	87 -150
Fasting	7	116	20	97 -150
Non-fasting	7	118	28	87 -140

* Conversion factor ug/100 ml to umol/l: 0.153

Detailed studies on zinc concentrations in infancy and childhood have been published by Henkin et al.(14) and by Kasperek et al.(15) who used capillary blood collected at various times of the day.They determined the zinc content by neutron activation. Both authors reported a slightly increasing trend in zinc values in infancy. This was almost simultaneously confirmed by the Denver group (16).

Kasperek reported a serum zinc value of 82 ± 15 ug/100 ml for infants less than 1 year,97 ± 20 ug/100 ml for children from 1 to 5 years,100 ± 16 ug/ 100 ml for children from 5 to 12 years and 117 ± 17 ug/ 100 ml for adults.The values found in our own laboratory for serum zinc concentrations in children as well as in adults are compatible with these literature data (14, 15, 16, 17).

Minor changes of the serum zinc levels after feeding have been described . This is particularly true if one compares each individual's levels in a fasting and non fasting state.Taken as a group however ,these variations remain within the limits of the reference range. They do not reach pathological values as described in clear cut deficiency syndromes (- 25 % of the lower limit).

Variations of zinc values according to circadian rythm and stress were described several years ago (18). In a marginal state of zinc deficiency, one single assay is usually insufficient to establish the diagnosis with certainty .A combination of different data will be required .Provided the sample is not hemolysed or contaminated, the plasma zinc level remains a very useful parameter for the assessment of the zinc status (13).

Serum zinc levels are reported to be approximately 16 % higher than those found in plasma .This was extensively investigated by Foley et al.(19,20) who attributed the effect to the aggregation of the platelets with subsequent release of zinc during clotting. These data were not confirmed by other investigators (17,22).

Sources of contamination should systematically been sought for and eliminated. To cite just a few: the rubber stoppers of vacutainer tubes (21,22),leaching their zinc content into the sample to be tested ,and all the zinc containing creams as Inotyol® and Calamine®.

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CHAPTER III. NUTRITIONAL ASPECTS.

3.1 LONGITUDINAL STUDY OF THE SELENIUM CONTENT IN HUMAN BREAST MILK IN BELGIUM

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ABSTRACT

The selenium content of expressed human milk obtained at different stages of lactation from 32 lactating mothers was measured by hydride generation atomic absorption spectrometry after previous wet acid digestion. The highest selenium level was found in colostrum (14.8 ng/g; wet weight) subsequently the content declined and levelled off after one month (9.4 ng/g; wet weight).

The daily selenium intake for Belgian infants of 3 months of age was found to be 7.1 ug (girls) and 8.1 ug (boys), which is lower than the values obtained in most other countries and lower than the recommended safe and adequate daily intake of 10 to 40 ug/day for the same age group.

Key words: Selenium, breast milk, infant intake.

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There has been an ever increasing interest in the role of selenium in human nutrition (1).Low selenium intakes have been associated with Keshan disease, a juvenile cardiomyopathy that occurs in certain parts of China (2,3); and various signs of selenium-responsive symptoms have been reported in patiens undergoing long term feeding with intravenous solutions that are pratically devoid of selenium (4-6). Moreover, epidemiological studies have suggested that low selenium intakes may be related to an increased incidence of cancer (7,8) and cardiovascular disease (9).

Breast milk is the only source of selenium for breast-fed infants. Although some reports on selenium content of breast milk are available in the literature (10-20),longitudinal studies of selenium content of breast milk are scarce (21-25) and no data for the Belgian population are available hitherto.

The purposes of this study are (1) to estimate the selenium content of breast milk obtained from Belgian lactating women at different periods of lactation, (2) to compare the obtained data with literature values and (3) to estimate the daily intake of these breast-fed infants.

MATERIALS AND METHODS

Sampling

Thirty-two healthy Belgian women, aged between 17 and 37 years, donated milk samples, obtained by expression, during the first six months of 1983. All subjects had delivered healthy, fullterm infants and did not experience any complications during pregnancy or at delivery. They had been living for at least one year in the surroundings of Antwerp and they were sampled at different times.

Colostrum milk was taken during the first three days, while transitional milk was sampled 5 up to 10 days post partum. Mature milk was sampled after 1 and 2 months of lactation.
From 14 mothers three or four samples were available, while the others provided only one or two samples. Sampling occurred in the morning before the infants were due to be fed.Approximately 10 ml was obtained in acid-rinsed glassware and store in polyethylene tubes.

ANALYSIS

For the selenium determination a hydride generation atomic absorption spectrometry with a Perkin-Elmer 372-MHS-1-unit, a high-purity quartz tube, closed at both ends by quartz windows, was used for atomization. Conditions for the evolution of hydrogen selenide have been described elsewhere (26). A nitric-perchloric acid digestion was previously carried out on about 10 ml samples, according to a procedure recommended by Robberecht and co-workers (27).
Throughout the method of standard addition was used for the calculation of concentrations.

RESULTS AND DISCUSSION

The selenium content of 73 milk-samples, taken at different stages of lactation, including one sample of pooled breast milk, are summarized in table 1.
As can be seen the selenium content of expressed human breast (foremilk) from Belgian mothers decreased with increasing time of lactation and levelled off on a concentration level of about 9.4 ng/g. No comparison with hindmilk can be made since only foremilk was sampled.

Distribution patterns revealed that 72% of the colostrum milk had a concentration level between 10 and 19.9 ng/g; 75% of the transitional milk had a selenium content varying between 5 and 14.9 ng/g, whereas 65% of the mature milk showed a concentration between 5.0 and 9.9 ng/g.Two milk samples,obtained after 2 months of lactation revealed distinctly higher concentrations, 14.4 and 15.4 ng/g respectively.

Individual longitudinal studies of the selenium content of human breast milk revealed that not all subjects showed the general pattern of decreasing selenium content. Probably food consumption habits or other factors regulate the milk selenium content. Shearer and Hadjimarkos (17) claimed that the selenium concentration in human milk reflected a different selenium intake via food, whereas Levander and coworkers (18) were unable to find any correlation between the selenium content in the breast milk and the dietary selenium intake by the mother. For Finland Kumpulainen and coworkers (25) proved that imported grain from the USA with high selenium content may be responsible for the different curves obtained in a longitudinal study of human breast milk samples.

Literature data on changes in selenium content of human breast milk samples during the lactation period are summarized in table 2, while table 3 contains additional data on selenium content of other breast milk samples. Specification of the samples is included in the table, as far as it is mentioned in the references.

All concentrations were converted to ng/ml by using a density of 1.034 (28) or a mean dry to wet ratio of 0.12 (15,21,28). All the data in table 2 show a significant decrease of the selenium content of the milk with increasing time of lactation and a levelling off at one month.

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Table 1. Selenium content (ng/g; wet weight) of human breast milk in Belgium.

Type of milk	Lactation time (days post partum)	n	Selenium concentration		
			mean ± SD	median	range
Colostrum	0 - 3	24	14.8 ± 4.9	15.0	6.6-27.2
Transitional	5 - 7	13	12.3 ± 4.4	11.0	5.5-18.7
	8 - 10	11	12.7 ± 3.5	12.6	6.7-17.7
Mature	30	15	9.4 ± 2.0	8.9	6.7-12.7
	60	9	9.9 ± 3.4	9.0	5.6-15.4
Pooled human milk		1	9.3	-	-

Table 2. Longitudinal changes of the selenium content (ng/ml) of human breast milk.

Sampling location (year)	n	Colostrum (0-3 days)	n	Transitional milk (4-10 days)	n	Mature milk	Specification	ref
New Zealand (1972)	6	26 ± 10 (12-35)	16	13 ± 4				10
West Germany (1978)	3	84 (65-108)	25	30.5 (16-53)	44	28.3 (11-54)	11-173 days	21
Greece (1978)	15	48 (33-69)	15	16 (10-20)	5	15 (11-22)	1 month	22
USA (1982)	18	41.2±17.3	-	-	8	18.0±3.8	1 month	23
					8	15.7±4.6	2 months	23
					8	15.1±5.8	3 months	23
Japan (1983)	7	80* (35-152)	10	29* (15-79)	9	18* (9-39)	1 month	24
					8	17* (6-28)	3 months	24
					7	18* (9-33)	5 months	24
Belgium (1983)	24	15.3 ± 5 (7-27)	24	12.7 ± 4.5 (6-19)	15	9.7±2.1	1 month	this
					9	10.2±3.2	2 months	study
					1	9.6	pooled mature milk	

* median value

Table 3 Literature data on the selenium content (ng/ml) of human breast milk.

Sampling Location	Concentration level n	mean ± SD	range	Specification	Ref.
Australia	40	12 ± 2		mature milk	11
Greece (Athens)	24	21 ± 1	-	29-195 days post partum	12
Italy	130	13.8± 0.9	1 - 51	21 mothers,15 days post partum	13
Japan	13	22.5 ± 4.2		mature milk	14
Scotland (Glasgow)	25	31 ± 10			15
Spain (Barcelona)	8	11.4 ± 3	9 - 18	3-10 days post partum	16
USA (17 states)	241	19 ± 0.4	7 - 62	17-869 days post partum	17
(Beltsville)		20 ± 4		1 month post partum	18
		15 ± 3.5		3-6 months post partum	18
(New York)		56		colostrum	19
		31			19
Yugoslavia (Ljubljana)	27	11.5 ± 3.6	5.7 - 16.7	0-14 days post partum	20

Table 4. Estimated daily intake of selenium (ug/day) for infants

Country	Average Se intake	Specification	ref
		breast milk, third month	
Belgium	8.1	840 ml/day (boys)	this study
	7.1	740 ml/day (girls)	this study
Germany (West)	22	breast milk, 2 month	21
	8	cow's milk, infant formula second month	21
Japan	17	breast milk, first 5 months	24
New Zealand	5	breast milk, first month	10
	2	bottle-fed cow's milk first month	10
Spain	4,5	breast milk, 5-6 months	16
	7	bottle fed cow's milk, 5-6 months	16
U.S.A.	10	breast milk, third month 683 ml/day	23

3.2 DAILY INTAKE OF SELENIUM OF BOTTLE FED INFANTS IN BELGIUM.

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ABSTRACT

The selenium content of commercial infant formula, processed milk and beikost was measured by hydride generation atomic absorption spectrometry after previous wet acid digestion. The median daily intake of infants (1-6 months) fed milk, soja or cereal based infant formula was 3.0-7.8 ug/day; this is lower than the mean daily intake of Belgian breast-fed infants (6.1-8.6 ug/day) and cow's milk bottle-fed infants (7.6-10.8 ug/day).

Key words: selenium, infant formula, cow's milk, daily intake.

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INTRODUCTION

Interest in the role of selenium in human nutrition is increasing as more and more investigators realize the essentiality of this trace element for human health (5,8,18).

The clinical features of human selenium deficiency are not yet characterized. There have been reports on low plasma selenium levels in patients receiving total parenteral nutrition for a long time (4) and in children living on artificial diets, providing only 1.0-4.7 ug Se/day (9,12,13). Low serum concentrations have been reported for premature infants with severe respiratory distress. After feeding a regular diet with a high selenium concentration serum concentrations returned to normal levels (1).

The Food and Nutrition Board (USA) has proposed adequate but safe intakes of selenium for infants (0-1 year): 10-60 ug/day; children (1-6 year): 20-120 ug/day; children (older than 6 years) and adults: 50-200 ug/day (9).

Daily intake by Belgian breast-fed infants has been determined by analysing the selenium content of human breast milk in Belgium (16). Since breast feeding is often replaced by artificial food, based on cow's milk or infant formula, the purposes of this study are (1) to clarify the selenium content of infant formula, cow's milk and beikost (2) to estimate the daily intake of artificially fed infants and (3) to compare the obtained data with literature values.

MATERIALS AND METHODS

1. Sampling

Twenty-five samples of commercial infant formula were obtained from the Antwerp Children's Hospital.

Eleven samples of processed cow's milk, sixteen samples of milk products, nine samples of strained baby food and five samples of cereal infant formula were purchased from local stores.

Ten samples of freshly prepared fruit porridge were obtained from different children gardens in Brussels.

Perishable samples were stored in a deep-freezer at -20 C, prior to analysis.

2. Analytical method

Hydride generation atomic absorption spectroscopy with a Perkin-Elmer 372-MHS-1-unit and a high-purity quartz tube, closed at both ends by quartz windows, for atomization was used for the selenium determination. Conditions for the evolution of hydrogen selenide have been described elsewhere (21). A nitric-perchloric acid digestion was previously carried out on about 10 ml or 1 g samples, according a procedure recommended by Robberecht and coworkers (15). The method of standard addition was used for concentration calculation through-out.

Triplicate analysis of the milk powder reference material IAEA-A gave a mean selenium concentration of 31.8 $\pm$ 2.5 ng/g.The concentration, recommended by the IAEA with an "acceptable" degree of confidence is 33.9 $\pm$ 7.2 ng/g. Triplicate analysis of the milk powder reference material NBS 1549 gave a mean selenium concentration of 106 $\pm$ 5 ng/g. The concentration, recommended by NBS is 110 $\pm$ 10 ng/g.

RESULTS

The results of the analysis of different infant formula are summarized in table 1. The formula are classified according to Roelandts (17). Three major classes are distinguished: milk based formula, soya based formula and milk formula for therapeutic use.

The milk based formula are subdivided in three classes: humanised milk for prematures and very young infants, soft milk, with a composition that approaches the composition of cow's milk and acidified milk, easier to digest through acidification of the proteins. Soya based milk is mostly used in cases of intolerance for proteins of cow's milk.

The content is given in terms of ready for use liquid (ng/ml) or caloric intake (ng Se/kcal) to allow mutual comparison between powder and liquid formula and calculation of mean daily intake.

The powdered milk and soya based infant formula have a mean dilution factor of 15 $\pm$ 1% and a mean caloric value of 0.7 $\pm$ 0.11 kcal/ml (n=13). A mean concentration of 5.3 $\pm$ 4.1 ng Se/ml and a median value of 4.7 ng Se/ml are calculated for the milk based infant formula while in terms of caloric intake a mean value of 8.0 $\pm$ 6.1 ng Se/kcal and a median concentration of 6.5 ng Se/kcal are obtained The formula for therapeutic use after appropriate dilution, have a mean concentration of 9.9 $\pm$ 9.3 ng Se/ml and a median value of 8.5 ng Se/ml , while in terms of caloric intake they revealed a mean value of 12.9 $\pm$ 12.2 ng/kcal and a median concentration of 12.3 ng Se/kcal.

As human milk usually serves as the standard of comparison for the nutritional adequacy of commercial infant formula, the selenium content of mature human milk in Belgium has been determined in an

earlier study (16).

The selenium contents of eleven milk samples are summarized in table 2. Processed milk has a mean selenium concentration of 5.9 ± 0.3 ng/ml when a 1/3 dilution of cow's milk for infant use is taken into account ,providing 19 ± 5.1 ng/kcal.

The selenium content of thirty-five samples of beikost are summarized in table 3, together with five samples of cereal infant formula. The mean selenium content in caloric terms of cereal infant formula is 9.7 ± 8.1 ng/kcal.

Table 4 reviews the selenium content of the different infant food classes in Belgium.

DISCUSSION

The mean 5.3 ng/ml as well as the median value 4.7 ng/ml of the selenium content in milk based infant formula after appropriate dilution are lower than the mean value of 9.9 ng/ml for mature human milk in Belgium (16). Only one milk infant formula has a higher selenium concentration than the mature human milk, namely Beba of Nestle which contains 13.6% maize.

The mean selenium content 9.9 ± 9.3 ng/ml of milk formula for therapeutic use is the same as the mean selenium content of mature human milk, which is merely due to the high concentration found in the Realmentyl formula. The median selenium content (8.5 ng/ml) and the selenium content in caloric terms (12.9 ± 10.2 ng/kcal) are lower than in the corresponding values for mature human milk in Belgium.

To calculate the mean daily intake of selenium by infants, we used two sets of mean caloric intake. The first set was used to calculate the daily intake at 3 to 6 months as presented by Whitehead et al. (22) who made a statistical analysis of energy intake data

collected in Cambridge, (U.K.) and data derived from a literature survey of cross-sectional food energy intakes of infants from Canada, Sweden, the U.K. and the U.S.A.. The second set was used to calculate the daily intake at one month and was taken from Ziegler and Fomon (24) who carried out a food intake and growth study. They found a value of 342 kcal/day ± 80 for infants of 2-3 months, which corresponded very well with the value 480 to 580 kcal/day claimed by Whitehead et al. (22).

The mean caloric intakes at 1,3 and 6 months of age were combined with the median selenium content and the range in selenium content of milk, soya and cereal infant formula, cow's milk and human milk. The results are summarized in table 5. A possible influence of the difference in energy density of the food consumed on the intake has not been taken in account. The recommended intake of 10-40 ug/day (6) is met only by the median cow's milk intake (10,8 ug/day) at six months of age, and approached by the median human milk, the median milk infant formula for therapeutic use and the soya based formula at six months of age (7.8-8.6 ug/day). The median intake by milk based infant formula (4.2 ug/day) and cereal infant formula (5.3 ug/day) at six months of age is a factor two too low. Since great variability of selenium concentrations exists for the different foods, individual formula may give an adequate intake of selenium. Of the milk infant formula, only the Beba and Realmentyl formula will follow up the recommendations of the Food and Nutrition Board, while only a few others approach the recommendations for children aged six months.

From table 3 it can be seen that introduction of other milk products will mostly not improve the selenium intake by infants, because only yoghurt has a higher selenium content compared with human milk.

In table 6 we compared our values with literature data. All literature data,except one (3),prove that the selenium intake of infants, fed commercial infant formula are lower than the selenium intake of breast-fed infants.

Since different reports (10,20) claimed that serum selenium concentration is age-dependent with a decrease in early infanthood, commercial infant food can lead to a further drop in serum selenium concentration, especially in some clinical cases (1).

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Table 1. Selenium content of processed milk

Manufacturer	Product	kcal/ml (a)	Selenium content ±SD(n) ng/ml (a)	ng/kcal
1.Milk based formula				
1.1 Humanised milk				
Guigoz	Nativa	0.70	1.3± 0.8(2)	1.9
	Nativa 1	0.70	0.6± 0.4(3)	0.9
Milupa	Neomil(c)	0.76	4.8± 2.2(3)	6.3
Nestle	Alprem(c)	0.70	4.6± 2.2(3)	6.6
	Almiron	0.77	4.9± 0.4(3)	6.4
	Almiron	0.77	2.3± 0.4(2)	3.0
	Almiron M2	0.77	3.9± 0.1(3)	5.1
1.2 Soft milk				
Nestle	Beba (b)	0.71	16.8± 3.1(3)	23.7
Nutricia	Nutrimel	0.34	4.6± 2.2(4)	13.5
1.3 Acidified milk				
Nestle	Pelargon	0.77	7.3± 0.8(3)	9.5
	Prodieton	0.62	6.3± 1.3(3)	10.2
Nutricia	Bebiron	0.72	6.0± 2.3(3)	8.3
2.Soya based infant formula				
Nutricia	Nutri-soya	0.74	8.9± 2.3(2)	12.0
3.Milk infant formula for therapeutic use				
De Bouronville	Biosorb Drink	1	5.8± 4.6(2)	5.8
Eaton	Vivonex	1	7.7± 2.7(3)	7.7
Mead Johnson	Nutramigen	0.69	8.5± 2.4(3)	12.3
	Pregestimyl	0.67	9.7± 1.8(7)	14.5
Nestle	CFI	0.37	12.8± 2.6(3)	34.6
	Al 110	0.67	10.3± 0.9(3)	15.4
	Alfare	0.79	4.7± 1.1(3)	5.9
Nutricia	Pepti-Junior	0.70	8.8± 0.7(4)	12.6
	Amirige	0.036	2.7± 0.4(3)	75.0
	Nutri 2000	1.0	1.3± 0.4(3)	1.3
Roussel	Presondyl	0.7	2.8± 0.4(2)	4.0
	Realmentyl	1.3	36± 5.0(2)	27.7

(a) ready for use liquid after appropriate dilution
(b) powder contains 13.6% maize
(c) for clinical use only

Table 2. Selenium content of processed milk

Sample	Number of samples	kcal/ml	Selenium content ng/ml	ng/kcal
Whole milk grade(UHT)	3	0.6	8.7 ± 0.8	15
Full cream milk(UHT)	2	0.6	9.5 ± 0.7	16
Semi skimmed milk(UHT)	4	0.45	8.4 ± 1.7	19
Skimmed milk(UHT)	2	0.34	8.8 ± 1.1	26

Tabel 3. Selenium content of cereal infant formula and beikost

Sample	Number of samples	kcal/g	Selenium content ng/g	ng/kcal
1. Cereal infant formula		(a)	(a)	
Mixed cereals based				
Guigoz-Florigor	2	3.9	44± 10	11.3
Nestle-Sinlac	2	3.9	14± 4	3.6
Nutricia-Bambix	2	3.7	84± 23	22.7
Rica based				
Nestle-Cerelac	2	4.4	36± 11	8.2
Wheat based				
Nestle-Cerelac	2	4.3	11± 3	2.6
2. Beikost		(b)	(b)	
Skimmed cheese	8	1.4	22± 7	16
Semi-skimmed cheese	5	1.2	19± 8	16
Yoghurt	3	0.6± 0.3	17± 9	37± 34
Fruit porridge	10	0.9± 0.15	8.9± 6.8	9.9± 7.7
Strained baby foods	9	0.67± 0.17	10± 6	15

(a) dry weight

(b) wet weight

Table 4 Selenium content of different infant food classes in Belgium

Type of food	n	selenium content ng/ml(a)	mean ± SD median range ng/kcal(a)
Milk based formula	12	5.3± 4.1 4.7 0.6-16.8	8.0± 6.1 6.5 0.9-23.7
Soya based formula	1	8.9± 2.3	12.0
Milk infant formula for therapeutic use (b)	11	9.9± 9.3 8.5 1.3-36	12.9± 10.2 12.3 1.3-34.6
Mature human milk(c)	24	9.9± 2.6 9.3 5.8-15.9	14.1 13.2 8.3-22.7
Processed cow's milk	11	5.9± 0.3 9.0 6.4-10.0	18± 5 16.6 13.3-27.9
Cereal infant formula	5	38± 29(d) 36 11-84	9.7± 8.1 8.2 36-22.7

(a) ready for use liquid after appropriate dilution, for processed cow's milk a 1/3 dilution was taken
(b) amerige not included, because it is used as supplement (1g for 100 ml)
(c) reference 16
(d) selenium content in ng/g dry weight

Table 5. Mean daily selenium intake for Belgian infants (ug/day)

Type of food	Months of age (caloric intake in kcal/day)		
	1[a](460)	3[a](545)	6[a](650)
	Daily selenium intake mean (range)		
Milk based formula	3.0 (0.4-10.9)	3.5 (0.5-12.9)	4.2 (0.6-15.4)
Soya based formula	5.5	6.5	7.8
Milk infant formula for therapeutic use	5.7 (0.6-15.9)	6.7 (0.7-18.9)	8.0 (0.8-22.5)
Mature human milk	6.1 (3.8-10.4)	7.2 (4.5-12.4)	8.6 (5.4-14.8)
Processed cow's milk	7.6 (6.1-12.8)	9.0 (7.2-15.2)	10.8 (8.6-18.1)
Cereal infant formula	- -	4.5 (2.0-12.4)	5.3 (2.3-14.8)

(a) from reference 23, 24.

Tabel 6. Estimated daily intake of selenium (ug/day) for infants

Country	Average intake	Age (months)	Specification	ref.
Belgium	7.2	3	breast milk	this study
	3.5	3	milk based formula	this study
	9.0	3	bottle-fed cow's milk	this study
	4.5	3	cereal infant formula	this study
Germany (West)	22	2	breast milk	11
	8	2	cow's milk	11
Japan	17	5	breast milk	7
New Zealand	5	1	breast milk	14
	2	1	bottle-fed cow's milk	14
Scotland	35	3	breast milk	2
	18	3	milk infant formula	2
Spain	4.5	5-6	breast milk	3
	10	5-6	milk infant formula	3
	7	5-6	bottle-fed cow's milk	3
USA	10	3	breast milk	19
	7	3	milk infant formula	19
			formula diets (700 kcal/day)	
	32	0-6	meat-based	23
	13	0-6	casein-based	23
	12	0-6	milk- and soya-based	23
	9.5	0-6	soya-based	23
	8.5	0-6	milk-based	23

Hospital Practice

ZINC CONTENT OF INTRAVENOUS SOLUTIONS

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Summary The zinc, copper, and selenium content of commonly used intravenous solutions, aminoacid solutions, and fresh-frozen plasma was determined by atomic absorption spectrometry and fluorimetry. Very small amounts of copper and variable, but substantial, amounts of zinc were present in all solutions tested. Zinc contamination could have come from the rubber stoppers for the glass bottles. Selenium could not be detected in any of the solutions. Fresh-frozen plasma contained high levels of zinc and physiological amounts of copper and selenium.

INTRODUCTION

BECAUSE zinc and copper deficiency were reported in patients on total parenteral nutrition (T.P.N.) for prolonged periods,[1-5] it has been repeatedly advocated that aminoacid solutions be supplemented by trace metals.[3-7] Often the tacit assumption is that these solutions contain only very small amounts of essential trace elements. We report here the zinc, copper, and selenium content of intravenous solutions commonly given to patients on T.P.N.

MATERIALS AND METHODS

Several bottles of each of various intravenous solutions were chosen at random from the Children's Hospital pharmacy. Each bottle was carefully opened and sampled to avoid contamination. Fresh-frozen plasma being given to patients on T.P.N. was sampled at random.

Seven rubber stoppers, used for glass bottles containing different intravenous solutions, were weighed and cut into pieces with a zinc-free surgical scalpel. A piece of each stopper was weighed, soaked, and heated to 200°C for 2 h in nitric acid of high purity (Merck, 65%). The zinc and copper content of the solutions so obtained was determined in duplicate by atomic absorption spectrometry. The selenium content was determined in duplicate by the fluorimetric method of Watkinson.[8]

RESULTS

The zinc, copper, and selenium content of aminoacid solutions, dextrose 5%/NaCl 0·45%, NaCl 0·9%, sterile water, and fresh-frozen plasma is shown in the table. Zinc levels were the most inconsistent of the elements studied, the variability being high not only between but also within batches. The highest zinc levels were found in glucose-containing or fructose-containing aminoacid solutions.

All rubber stoppers contained a significant amount of zinc. Values ranging from 9·2 to 29·4 with a mean of 15·2 mg zinc/g rubber were found. The results were fairly consistent for a given manufacturer.

DISCUSSION

This study shows that most intravenous solutions commonly given to patients on T.P.N. contain very low levels of copper, minute quantities of selenium, but substantial amounts of zinc, and that fresh-frozen plasma contains high zinc levels and physiological levels of copper and selenium. Since normal values in our laboratory for serum zinc and copper and for plasma-selenium are 117±20 μg/dl, 122±22 μg/dl, and 9·8±1 μg/dl respectively, plasma infusions must be considered a source of essential trace metals.

The high zinc content found in the rubber stoppers used in the glass bottles confirms the findings of Jetton et al., who have shown that zinc can leach out of these stoppers into the solution.[9] Theoretically, the extent of

TRACE ELEMENT CONTENT OF INTRAVENOUS SOLUTIONS

Solutions	No.	Zinc (μg/dl)		Copper (μg/dl)		Selenium (μg/dl)
		Mean	Range	Mean	Range	
TPN solutions						
'Vamin glucose' (Vitrum)	5	81·5	67–102	3·8	3·5–4·2	<0·1
'Vamin fructose' (Vitrum)	2		39–43		3–4	<0·1
'Vamin N' (Vitrum)	4	57	29–98	3·3	3–4	<0·1
'Aminess' (Astra Ned)	3	10	5·8–17	1		<0·1
'Trophysan' (Egic)	2		57–153		ND†–4	<0·1
*Others**						
Dextrose 5%/NaCl 0·45%	4	27	4–56	2·7	2·3–3	
NaCl 0·9%	3	6·5	7·4–9	8	6·1–9	
Sterile water	3	2·6	1·7–3·3		ND†	
Fresh-frozen plasma‡	5	191 (±15)		113 (±20)		7·7 (±0·6)

*All solutions were obtained from the same manufacturer.
†Not detectable by the method used.
‡Results for fresh-frozen plasma given as mean±S.D.

contamination depends on variables such as duration of exposure of the solution to the stopper and shaking during transport and storage; these can largely account for the inconsistent zinc levels. The largest amounts of zinc were detected in the carbohydrate-containing aminoacid solutions probably because factors specific to these solutions, such as aminosugar complexes, slowly formed by the Maillard reaction and well known chelators,[10] may increase the amount of zinc leached out.

The lowest amount of trace element contamination was observed in aminoacid solutions. The copper content was negligible in all solutions tested. Greene[2] reported 2–7·2 μg zinc/dl 'Freamine' and Okada[4] 2–8 μg zinc/dl 'Ispol' 12% and 'Ami-U'. We found very low zinc levels in 'Aminess' but James et al.[11] found 117·5±38·9 μg zinc/dl 'Aminofusin 850'. Protein hydrolysates also contained high levels of zinc—Jetton et al. found 300 μg zinc/dl 'Hyprotigen'[9] and Greene reported 14–60 μg zinc/dl 'Aminosol' and 'Amigen'.[2] These protein hydrolysates, as well as the 'Vamin' solutions, could provide adults and infants on T.P.N. with their basal daily requirement (20 μg and 40 μg zinc/kg body weight respectively[12]), but may still be insufficient for some patients. It is thus imperative to know not only the trace element composition of intravenous solutions (it should be stated on the label) but also the zinc status of the patient, so that one could, if necessary, add trace metals to the solution in order to correct for previous deficits and for current gastrointestinal and urinary losses,[7] as well as provide for daily basal requirements.

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Selenium Status of Infants on Nutritional Support

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ABSTRACT. Van Caillie-Bertrand, M., Degenhart, H. J. and Fernandes, J. (Department of Paediatrics, Erasmus University, University Hospital, Sophia Children's Hospital, Rotterdam, The Netherlands). Selenium status of infants on nutritional support. Acta Paediatr Scand 73: 816, 1984.

We investigated the selenium status of 5 infants while on nutritional support. After 4 weeks of parenteral nutrition a significant fall in plasma selenium concentrations was observed (mean ± SD: 0.8±0.5 μg/dl; normal for this age: 3.6±0.9 μg/dl). In 1 infant the decline in selenium value occurred simultaneously with a transient rise in transaminases. A parallel but delayed decrease in red blood cell-glutathione peroxidase activity was seen in 3 patients. After reintroduction of enteral foods, the selenium levels increased progressively to and reached control values after 6 weeks, 4 and 5 months respectively in 3 patients, suggesting that the selenium requirement on TPN was not met. We consider it essential to provide longterm TPN patients with physiological amounts of selenium in order to prevent the progressive development of a deficiency state. ***Key words: Selenium, erythrocyte glutathione peroxidase activity, infants, nutritional support.***

In those patients on total parenteral nutrition (TPN) who receive little or no additional nutrition by mouth for months or even years, clinical deficiency states may appear that otherwise are rarely seen (1). The relevance of selenium deficiency was first suggested by Van Ry et al. (2), when they observed a patient on TPN who developed tenderness in the thighs with inability to walk. This syndrome responded to selenium supplementation alone. Later Fleming et al. (1), and Johnson et al. (3) described in similar patients a progressive cardiomyopathy very comparable to Keshan disease. Epidemiological studies in China had already shown that selenium supplementation prevents the occurrence of a fatal cardiomyopathy in children living in selenium deficient areas (4). The occurrence of a sudden cardiac arrest in 2 infants after 18 and 9 months of TPN respectively, prompted us to investigate the selenium status of 5 infants while on nutritional support.

PATIENTS AND METHODS

The selenium status of five infants was studied. They received TPN for periods ranging from 14 days to 10 months, thereafter supplemented with a naso-gastric drip with an elemental diet and oral foods. The indications for TPN were massive small-bowel resection (3), protracted diarrhea of infancy (1) and meningitis with severe malnutrition (1). The age at which TPN was begun ranged from 1 to 4 months. The regimen used for TPN was similar to that reported previously (5). The infusate consisted of three basic solutions: (1) Vamin, composed of amino-acids, glucose and electrolytes, (2) 10% Intralipid containing the fat emulsion, (3) 15% glucose. Daily requirements of vitamins and minerals were added to the basic infusions and fresh frozen plasma was given twice a week.

Age matched controls were 12 infants (0–12 months), all receiving a humanized cow's milk infant formula (Almiron®-Nutricia) who had to undergo minor operations, diagnostic procedures or were seen at a last follow-up visit after an acute illness. All were growing well and had a height and weight >P3 for age.

The glutathione peroxidase activity of the red cells was measured at 30°, using butylhydroperoxide as described by Paglia & Valentine (6). The plasma selenium concentrations were determined by the method of Watkinson (7).

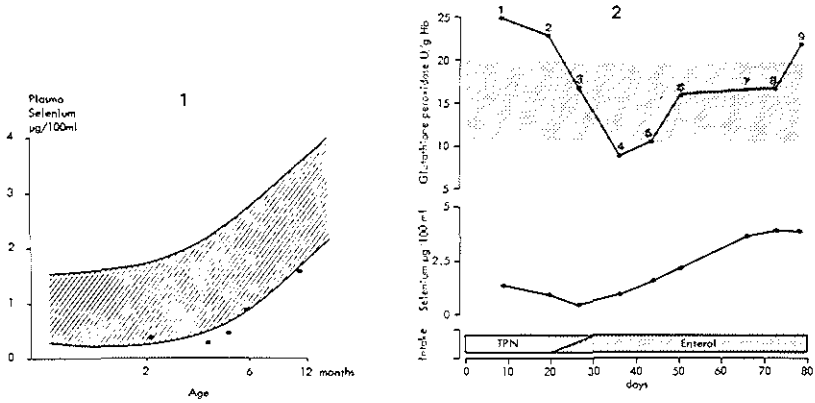

Fig. 1. Plasma selenium concentrations in infants after 4 weeks on nutritional support. The shaded area indicates the reference range.

Fig. 2. Selenium concentrations of plasma and glutathione peroxidase activity in erythrocytes in patient 2 during TPN and after reintroduction of enteral foods. Shaded areas indicate the reference range.

RESULTS

All five infants showed a satisfactory weight gain while on TPN. The plasma selenium concentrations during nutritional support (TPN followed by elemental diet and parenteral supplementation) are shown in Table 1 and Fig. 1. After the introduction of enteral foods the selenium levels rose progressively and in 3 infants reached normal levels after 6 weeks, 4 and 5 months respectively. In the fourth patient, the level had risen from 0.4 µg/dl to 1.5 µg/dl 4 weeks after discharge when he was lost to follow up. The fifth infant who received first an elemental diet and thereafter a Soya feed, had 4 weeks after resuming oral intake still a very low selenium level: 0.4 µg/dl.

Table 1. *Plasma selenium levels in infants on nutritional support and age-matched control subjects*

	Plasma selenium concentrations (µg/100 ml)			
	Week of TPN			
	1	2	4[a]	On oral foods
Patients				
1	2.2	2.1	0.9	0.5
2	1.4	1.0	0.5	4.0
3	–	0.3	0.4	1.5
4	–	–	1.6	5.4
5	1.1	–	<0.4	5.6
Controls				
No. 12	3.6±0.9 (mean±SD; as shown in Fig. 1)			

[a] Supplemented with elemental diet.

A parallel but delayed decline in erythrocyte gluthatione peroxidase activity was seen in 3 patients: from 25 to 9 U/g Hb, from 34 to 15 U/g Hb, and from 17 to 14 U/g Hb. Although suggestive these values were not significantly different from those observed in age matched controls (mean ± SD: 16±4 U/g Hb). In infant 2, the erythrocyte glutathione peroxidase activity rose progressively with the selenium level after the reintroduction of enteral feeding (Fig. 2).

Liver function tests were periodically determined during TPN. Only in patient 1 we noted a transient elevation of the transaminases without hyperbilirubinemia. This coincided with the lowest selenium level in this patient.

DISCUSSION

We showed a progressive, steady and significant decline with time of plasma selenium values in 5 infants while on nutritional support. This occurred despite the weekly administration of fresh frozen plasma which contains trace amounts of this metal (8). The selenium content of the usual solutions given in TPN (9) is extremely low: it is consequently not possible to maintain in those patients plasma levels within age matched range without supplementation.

We confirmed the existence of a positive correlation between declining plasma selenium levels and glutathione peroxidase activities in the red blood cells (10). Moreover in patient 2, the glutathione peroxidase activity progressively rose with increasing selenium levels after reintroduction of enteral foods. This suggests that the selenium requirement was not met during the period on nutritional support.

It has been shown in China that an adequate selenium intake prevents the occurrence of the fatal cardiomyopathy known as Keshan disease. It has also been shown in animals that adequate selenium levels protects liver membranes against diquat and paraquat poisoning (11). The transient elevation of transaminase occurring simultaneously with the lowest selenium level in one of those five infants, as in the child reported by Kien & Ganther (10), is therefore disturbing. As an integral part of the enzyme glutathione peroxidase (12), selenium provides an efficient means of defense against the build-up of peroxides which otherwise are able to damage cell membranes and macromolecules (13). It is highly probable that a deficiency will not become apparent unless a coexisting vitamin E deficiency or a special stress (e.g. an infection) occurs.

We consider that one should not wait until clear-cut deficiencies have developed but should aim to maintain plasma levels within the physiological range, recognising the fact that selenium in man is age dependent. The amount that should be supplemented is difficult to define as the optimal requirements are not exactly known, while selenium in larger doses is toxic. A conservative estimation is 2 μg/kg/day for infants, 10–20 μg/day for children (14) and 50–100 μg/day for adults (1, 15).

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TRACE METALS IN CYSTIC FIBROSIS

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ABSTRACT. van Caillie-Bertrand, M., de Biéville, F., Neijens, H., Kerrebijn, K., Fernandes, J. and Degenhart, H. (Department of Paediatrics, Erasmus University and University Hospital, Rotterdam, The Netherlands). Trace metals in cystic fibrosis. Acta Paediatr Scand, 71:203, 1982.—Serum zinc and copper concentration, 24 hrs urinary zinc and copper excretion, plasma selenium and red blood cell glutathione peroxidase activity were measured in 13 cystic fibrosis patients aged 6 to 15 years. The mean serum zinc value ± S.D. (17.3 μmol/l ±4.6) did not differ from that of the control group (17.9 μmol/l ±3.1). Urinary zinc excretion in 12 out of 13 patients was within the normal range (1.53–13.8 μmol/24 hrs). The mean serum copper ± S.D. (23.8 μmol/l ±4.2) was not significantly elevated as compared to the value found in the control group (19.2 μmol/l ±3.5), but 4 children, including 1 with documented portal hypertension, showed an urinary copper excretion >0.94 μmol/24 hrs (normal: 0.16–0.80 μmol/24 hrs). Mean plasma selenium ± S.D. (0.84 μmol/l ± 0.25) was significantly reduced as compared to the control group (1.0 μmol/l ± 0.15) ($p<0.05$). The correlation between selenium concentration and RBC glutathione peroxidase activity was significant ($p<0.01$). A negative correlation was also found between plasma selenium and 24 hrs faecal fat excretion ($p<0.05$). It is concluded that CF children with severe dysfunction of the exocrine pancreas are at increased risk to develop symptoms of subclinical or manifest zinc and/or selenium deficiency. Appropriate supplementation should therefore systematically be considered.

KEY WORDS: Trace metals, zinc, copper, selenium, glutathione peroxidase, cystic fibrosis

Malabsorption is one of the main features of cystic fibrosis (CF). In spite of an adequate supply of calories, vitamins and pancreatic enzymes some patients fail to grow. There is now ample evidence that certain cases of nutritional dwarfism and retarded pubertal development are associated with zinc deficiency and that the administration of zinc normalizes growth and development (1). Not only zinc has been shown to be essential for man but also (among others) copper (2) and recently selenium (3). Animal models have shown that absorption of trace metals proceeds most efficiently in the proximal small intestine; probably mediated by specific ligands present in pancreatic secretion and/or mucosal cells (4). Children with exocrine pancreatic insufficiency, as observed in CF, might therefore have impaired absorption and secondary deficiency of these essential micronutrients. The purpose of this study was then to determine whether children with CF are at risk to develop deficiency of zinc, copper and selenium.

MATERIALS AND METHODS

Thirteen patients with CF, aged 6 to 15 years were studied. While on a high protein, moderate fat intake all had severe steatorrhea and therefore received pancreatic replacement therapy. Eleven patients had failure to thrive, 6 had severe pulmonary disease. Only 1 had documented portal hypertension; 12 other patients did not have clinical evidence of liver disease and none was jaundiced.

Zinc and copper concentrations in serum, 24 hours urine and pancreatic preparations were measured in duplicate by atomic absorption spectrometry. Plasma selenium concentrations and selenium content of pancreatic preparations were determined by the fluorimetric method of Watkinson (5). The glutathione peroxidase activity in the red blood cells (RBC) was measured after haemolysis, using the coupled test procedure with butylhydroperoxide as substrate as described by Paglia & Valentine (6). Fae-

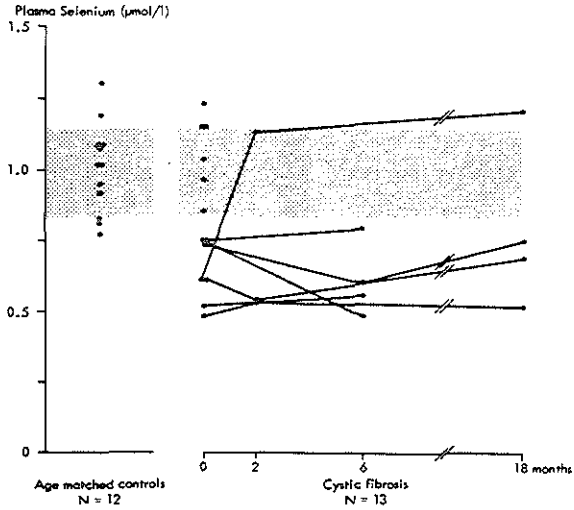

Fig. 1. Plasma selenium concentrations in CF patients as compared to age matched controls. ▩ indicates normal range (mean ± S.D.).

cal fat excretion was determined in duplicate during two collection periods of 72 hours and the results were averaged for each patient. For statistical analysis the Student's *t*-test and, where appropriate, the Spearman rank correlation coefficient were used (7).

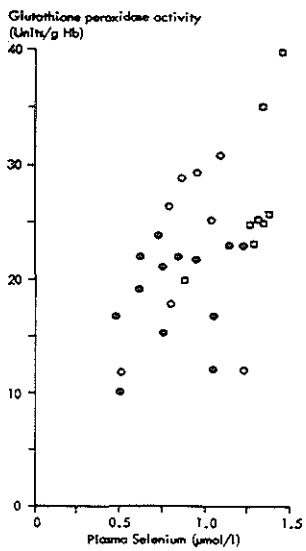

Fig. 2. Relationship between glutathione peroxidase activity and plasma selenium concentration. ●, CF patients; ○, age matched controls; □, normal adults. Spearman $\varrho=0.447$, $p<0.01$.

RESULTS

Although the mean serum zinc concentration ± S.D. (17.3 μmol/l ±4.6) found in the CF group did not differ significantly from that of a control group (17.9 μmol/l ±3.1), 3 patients had a serum zinc level less than 2 S.D. below the mean of the control group (<11.8 μmol/l). In one of the three the urinary zinc excretion was markedly elevated (18.4 μmol/24 hrs), in the other two and in the remaining patients it was within the normal range (1.53–13.8 μmol/24 hrs).

The mean serum copper concentration ± S.D. (23.8±4.2 μmol/l) was high, but not significantly elevated when compared to that of the control group (19.2±3.5 μmol/l). Four CF patients, however, including the one with portal hypertension showed a 24 hrs urinary copper excretion greater than 0.95 μmol, as compared to none of 11 children with noncholestatic chronic liver disease (normal range: 0.16–0.80 μmol/24 hrs). One CF patient approached an excretion of 2.0 μmol/24 hrs, an amount we have seen in a patient with presymptomatic Wilson's disease.

Plasma selenium values (mean ±S.D.: 0.84 μmol/l ±0.25) were significantly reduced as

Table 1. *Trace metal content of pancreatic preparations*

Name preparation		Selenium (μg/g)[d]	Zinc (μg/g)[d]	Copper (μg/g)[d]
Lyopase®[a]	Mean	0.42 (n=3)	44 (n=2)	1.1 (n=2)
	Range	–	–	–
Cotazym®[b] Forte	Mean	0.53 (n=5)	49 (n=6)	1.7 (n=7)
	Range	0.44–0.59	46–51	1.3–2.0
Pancreatic[c] grain	Mean	1.46 (n=12)	119 (n=8)	5.5 (n=8)
	Range	1.29–1.71	118–122	1.9–8.0
Pancreatic[c] capsule	Mean	0.29 (n=6)	34.2 (n=6)	1.2 (n=6)
	Range	0.20–0.42	27–40	0.8–1.4

[a] A. Christaens, Brussels.
[b] Organon, Oss.
[c] Several sources.
[d] The weight of pancreatic preparations varies considerably. For the commercial preparations Lyopase® and Cotazym Forte® the weight was found to be approximately 2 g per powder capsule, resp. 610 mg per tablet.

compared to the values found in age matched controls (mean ± S.D.: 1.0 μmol/l ±0.15) (p<0.05). All 7 children with a plasma selenium value <0.84 μmol/l were followed, 4 of them up to 18 months after the first investigation. All patients except one remained below the normal range (Fig. 1). In 29 blood samples (13 CF, 8 age matched controls, 8 adults) both the plasma selenium and the RBC glutathione activity were determined. In Fig. 2 the relationship between the two is shown. The correlation appeared to be highly significant (ϱ=0.447, p<0.01). In Fig. 3 the relationship between faecal fat excretion and plasma selenium concentration is shown. Five out of 6 CF children with a faecal fat excretion >15 g/24 hrs, had a plasma selenium value <0.84 μmol/l. The negative correlation between faecal fat excretion and plasma selenium concentration was significant (ϱ=0.587, p<0.025). The only child who normalized his plasma selenium concentration during the period of observation showed a simultaneous decrease in faecal fat excretion from 25 g to 6.5 g fat/day.

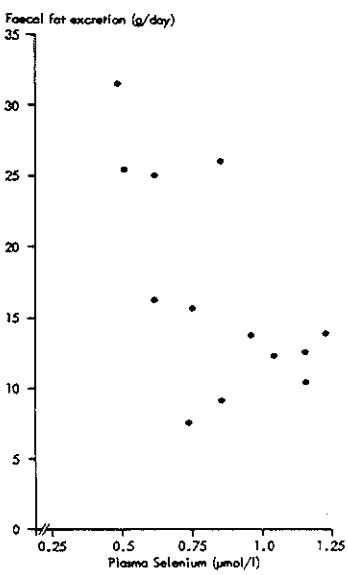

Fig. 3. Relationship between daily faecal fat excretion and plasma selenium concentration in 13 CF children. Spearman ϱ=0.587, p<0.025.

The trace metal content of the usual pancreatic preparations is shown in Table 1.

DISCUSSION

CF children with severe exocrine pancreas dysfunction can be expected to develop symptoms of sub-clinical and/or manifest trace metal deficiency. Appropriate supplementation adapted to the patients' needs should then be considered. In concordance with the reports of others (1, 8) we have found sporadically a lowered serum zinc value, although without

decreased urinary zinc excretion as would arise from zinc malabsorption. One patient even showed an increased urinary loss of zinc.

The finding of an increased urinary copper excretion in 30% of our CF children is disturbing. Further studies will have to show whether CF patients excrete sufficient amounts of copper with their bile as to prevent accumulation of copper in the liver. If not, it could increase the risk of secondary liver damage (9) and contribute to the development of liver cirrhosis, one of the late manifestations of CF.

Selenium, a trace metal essential for man (3, 10, 11, 12), appeared to be markedly reduced in 7 out of 13 GF children in this study. Selenium deficiency causes widely different symptoms in various species, the young being the most susceptible. In the chick, selenium deficiency results in "exsudative diathesis" and pancreatic atrophy, both subsiding with the administration of selenium (13). In the subhuman primate, prolonged deficiency causes loss of body weight, listlessness, loss of hair and leads eventually to death (14). Very little is known about selenium metabolism in man. Children with kwashiorkor have been reported to respond with increased weight gain on supplementation of the diet with selenium (15). In China, a cardiomyopathy known as Keshan disease, seems to be prevented with selenium (10, 20). A clinical response to selenium supplementation has also recently been described in a patient on total parenteral nutrition, who complained of tenderness in the thigh muscles with inability to walk (3).

Selenium functions as an integral part of glutathione peroxidase, an enzyme reducing (i.a.) toxic lipid peroxides. This enzyme has been found in almost every parenchymal tissue investigated. As such, it protects cellular membranes from the effects of peroxides (16). In animals and in man (17, 18) the selenium content of the diet affects the activity of glutathione peroxidase in the erythrocytes.

The low dietary intake observed in the Netherlands where the soil selenium content is particularly low might explain our findings, but not entirely. Indeed the selenium content of the usual pancreatic preparations appears to be unexpectedly high (from 0.2 to 1.7 μg per gram) providing CF children with a supplement of 10 to 20 μg selenium/day, which represents the daily selenium dietary intake of a New Zealand resident (12). The low plasma selenium concentration and reduced RBC glutathione peroxidase activity found in this study are most probably due to an impaired absorption of this metal in the presence of an excess of faecal fat. Supporting this view is the negative correlation between faecal fat excretion and plasma selenium levels.

The selenium status of the human individual may affect the ability of the organism to protect biomembranes against oxidative challenge (16). We are unaware of any data on selenium concentration and/or glutathione peroxidase activity in the parenchymal tissues of children with CF, although Lloyd-Still in a recent paper reported decreased blood levels in CF children (19). The fact that the activity of the human RBC glutathione peroxidase correlates closely with the selenium content in the diet (18), is indicative of the human requirement for this trace metal. Although selenium is a potentially toxic nutrient, supplementation in order to normalize plasma content may be justified on the conditions that plasma levels be monitored periodically.

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CHAPTER V. WILSON'S DISEASE

5.1 ASSESSMENT OF D-PENICILLAMINE THERAPY

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SUMMARY

Serum copper and zinc levels and 24 h urinary copper and zinc excretion were determined serially from the onset of therapy with D-Penicillamine (D-P) in 4 children with Wilson's disease. The data show a progressive decrease of both serum copper and zinc levels in all patients. The urinary copper excretion gradually levelled off to approximately 50% of initial values, whereas the zinc excretion increased. Consequently the urinary zinc copper ratios increased with the duration of therapy. Decoppering was considered to be adequate as soon as a challenge with a test dose of D-P did not entail an increase in copper excretion. As for zinc its urinary excretion was further stepped up by the test dose. Zinc depletion was suspected in one patient who developed clinical features of zinc deficiency while on D-P maintenance therapy. Lowering the dose of D-P alleviated the symptoms as the urinary zinc loss decreased from 4200 to 2200 ug/24h (64 to 19 umol/24 h), while the copper excretion remained largely unchanged.The data obtained indicate that D-P alters the metabolism of both copper and zinc.The extent of this phenomenon is not only dose dependent but also related to the efficacy of decoppering. In conclusion: one has to monitor both copper and zinc levels to assess the benefits of the therapy but also the risks of inducing manifest or subclinical zinc deficiency.

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The clinical manifestations of Wilson's disease are due to the accumulation of toxic amounts of copper in the liver, kidney, brain, cornea and other tissues. As first described by Walshe (1) and further confirmed by others (2), the treatment with D-Penicillamine (Dimethylcysteine), combined with a low copper diet, has markedly improved the outcome of the disease. The ultimate prognosis,however, still depends on the age, symptoms and degree of tissue damage at the time of diagnosis (3,4).

D-Penicillamine is an effective chelator of copper, is well absorbed from the gastrointestinal tract and promotes the urinary excretion of copper (1). Thus it allows the removal of the excessive amounts stored and it prevents further accumulation of copper. According to current recommendations (3,5) the treatment should be continued throughout life and the dosage adapted to the patient's tolerance. Practical guidelines are scanty however: a small dose might not be effective enough while a larger amount could be toxic (5). Other essential metals, especially zinc, may, by increased dosage, simultaneously be removed. To assess the efficacy of the therapy and avoid possible deficiency we studied therefore both zinc and copper values in serum and urine from the onset of D-P therapy in 4 children with the hepatic form of Wilson's disease.

PATIENTS

The age of the children in this study was 5,3,7 and 10 years, respectively, when D-P therapy was started. Criteria for diagnosis (3) were 1) liver copper levels >325 ug/g dry weight 2)urinary

copper excretion > 80 ug / 24h 3) abnormal radiocopper kinetics in patients 1 and 2 (no significant incorporation of ^{64}Cu in ceruloplasmin) 4)low serum ceruloplasmin levels (<20 mg/dl) 5)familial occurrence in patients 2, 3 and 4. None had Kayser-Fleischer rings or neurologic symptoms. Patient 1 presented with a chronic active hepatitis like syndrome, patient 3 with liver fibrosis, patients 2 and 4 were asymptomatic siblings of known patients with Wilson's disease. None was jaundiced.

THERAPEUTIC DESIGN

Soon after confirmation of the diagnosis of Wilson's disease the patients were treated with D-P and a low copper diet was prescribed. The drug was administered in 3 doses given with the meals. The initial dose was 500 mg daily, except for patient 1 who received 300 mg. This dose was maintained during the initial six months in patients 2, 3 and 4. After this induction period a challenge dose was given. This was equal to the maintenance dose +250 mg.
Dependent upon the results obtained, the maintenance dose was increased or continued unchanged.

METHODS

Liver function tests were carried out by standard techniques. Serum and 24 h urine for zinc and copper assays were collected in metal free containers at monthly intervals during the first year and at bimonthly intervals thereafter. All metal analyses were performed in duplicate by means of atomic absorption spectrophotometry.

RESULTS

CLINICAL COURSE: All children were asymptomatic with normalisation of the liver function tests at the last observation. They did not

develop any neurological signs of the disease nor Kayser-Fleischer rings. While receiving 1 g of D-P daily patient 1, however,developed skin lesions seven months after the start of therapy. These lesions consisted of alterations in hair texture with hair loss,parakeratosis of the extremities and deep grooves at the foot soles. Urinary zinc excretion at that moment was 4180 ug zinc/24 h (64.3 umol/24h). Reducing the D-P dosage to 750 mg, alleviated the symptoms while the 24 h urinary zinc excretion decreased to 2260 ug (34.8 umol), and the copper excretion remained largely unchanged (1200 ug/24 h) (19 umol/24 h).

COPPER AND ZINC DATA. Serum copper levels decreased steadily during D-P therapy: from 51 to 28 (8.0 to 4.4), 55 to 20 (8.7 to 3.1), 72 to 12 (1 1.4 to 1.9), 64 to <10 ug/dl (1 0.1 to < 15 umol/l) in patient 1,2,3,and 4 respectively. Serum zinc levels, initially high (mean: 175 ug/dl (27 umol/l), range 250 to 143 ug/dl (38 to 22 umol/l)) followed the same trend and reached the low normal range (mean: 83 ug/dl (1 2.7 umol/l), range: 79 to 87 ug/dl (12 to 1 3.3 umol/l)) during maintenance therapy. The lowest value (64 ug/dl (9.8 umol/l)) was seen in patient 3, 15 months after the onset of therapy.

Urinary copper and zinc excretions are summarised in fig.1. With the exception of patient 4, copper excretion exceeded zinc excretion, even on a relatively low dose of D-P (500 mg) at the onset of therapy.As treatment continued the urinary copper excretion decreased progressively to approximately 50% of initial values, whereas the urinary zinc excretion increased further. Consequently the urinary zinc copper ratios increased with the duration of therapy as show in the table.

At the end of the initial phase of treatment (thus usually after

more than six months therapy) a challenge dose of D-P resulted in a rise in zinc excretion. No further increase in copper excretion was then observed, suggesting that decoppering was adequate. During the initial phase of treatment, however, this challenge dose of D-P did cause a rise in copper excretion as shown in patients 3 and 4, 4 and 5 months respectively after the onset of therapy.

DISCUSSION

The data obtained show that D-P alters the excretion of both copper and zinc in patients with Wilson's disease. The extent of this phenomenon is not only dose dependent but also related to the efficacy of decoppering. A comparison of the dissociation constants for metal-penicillamine complexes shows a decreasing stability in the order Hg> Cu> Ag> Pb> Ni> Cd> Zn> Co> Fe> Mn (8). Consequently more copper is excreted than zinc in the copper loaded patient at the beginning of therapy. As the mass of copper decreases, zinc having a lower affinity constant but a relatively high abundance, will then be excreted in greater amounts.Therefore the appearance of increased zincuria during a D-P challenge is of significance both to assessing the benefits of the therapy and the risks of inducing zinc as well as other trace metals deficiencies.

The effectiveness of therapy is usually assessed by serial determinations of transaminases, serum copper and 24h urinary copper excretion (3,5,6).Our observation confirms previous reports: in children as well as in adults, serum copper falls with time, as the non-ceruloplasmin bound fraction of serum copper returns to normal (1,7). 24 h urinary excretion rises initially, but levels off to approximately 50% of the initial values ((600-1000 ug/24 h (9.5 - 15 umol/24 h)) during maintenance therapy.

Serial serum zinc and 24 h urinary zinc excretion, however, are not routinely measured. The figure shows that these determinations give important complementary information for assessing the adequacy of therapy. If decoppering has been effective the increase in zinc excretion is not accompanied by any significant increase in copper excretion when the patient is challenged by a larger dose of D-P . If the challenge dose steps up copper excretion, however,a higher maintenance dose of D-P is indicated. This approach may help to improve the effectiveness of the treatment and thus prevent a partial failure of the therapy.

Owing to its non-specificity as a chelator,D-P may also affect other biologically important metals. Although in clinical use for more than 25 years, few data exist about the long term effects of D-P upon the homeostasis of essential trace metals other than copper (9). Our findings support a previous report claiming that zinc deficiency may occur during treatment with D-P if urinary excretion exceeds the zinc absorption rate (10), although this was not substantiated with balance studies. Some of the known side effects of the drug strongly suggest a zinc deficiency: skin lesions on pressure points, desquamations, psoriatiform eruptions, delayed wound healing, alopecia and sometimes loss of taste acuity, glossitis and stomatitis (11). Some of the toxic effects of D-P may be attributable to hypersensivity, others to the fact that D-P is an important antimetabolite of pyridoxine or to its direct action upon collagen (12). A few are almost certainly due to zinc deficiency and possibly also to deficiencies of other essential trace metals.

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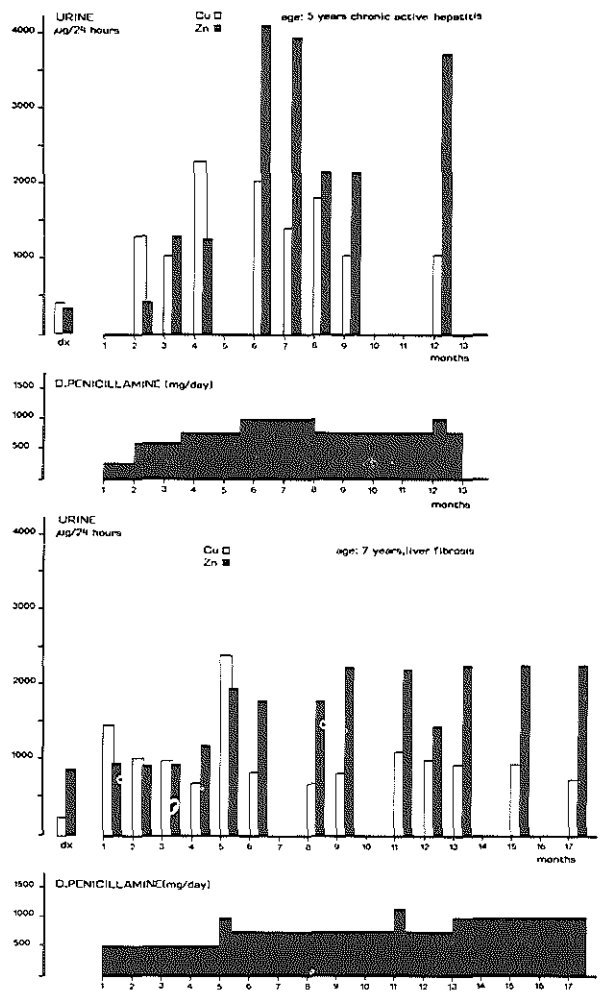
Fig.1 24 h urinary zinc and copper excretion in patients 1,2,3 and 4 according to D-Penicillamine dosage, from the onset of therapy and during steady state.

Conversion -traditional units to SI:copper 1 ug/24h = 0.016 umol/24h

zinc 1 ug/24h = 0.015 umol/24h.



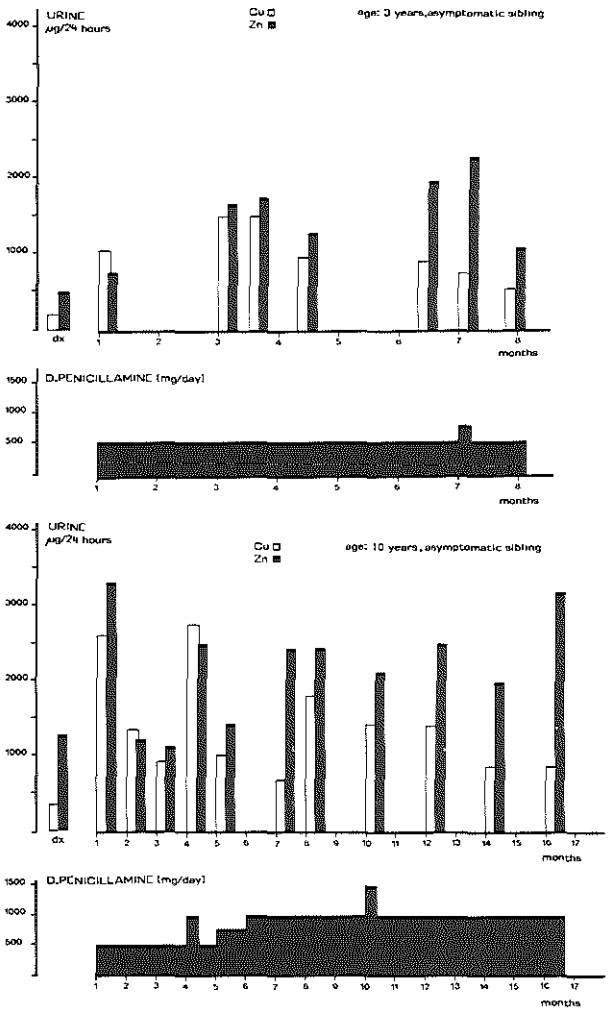

URINE
µg/24 hours
Cu
Zn
age: 3 years, asymptomatic sibling
4000
3000
2000
1000
dx
1
2
3
4
5
6
7
8
months
D-PENICILLAMINE (mg/day)
1500
1000
500
age: 10 years, asymptomatic sibling
9
10
11
12
13
14
15
16
17

TABLE 24 h Urinary zinc copper ratios at onset of D-Penicillamine therapy and during treatment

Patients	Start of treatment	(N)	After D-P challenge	Steady state	(N)	After D-P challenge
1	0.63	(3)	-	2.18	(1)	2.88
2	1.12	(4)	-	2.15	(2)	3.14
3	1.01	(4)	0.84	2.33	(6)	3.64
4	1.09	(3)	0.88	2.09	(16)	3.76
Mean ± SD	0.98 ± 0.4	(14)		2.17 ± 0.6	(25)	

5.2 ORAL ZINC SULPHATE FOR WILSON'S DISEASE

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SUMMARY

After initial decoppering with D-Penicillamine (D-P) the effect of oral zinc sulphate (3x150 mg/day as a loading dose, 3x100 mg/day as a maintenance dose) was evaluated after completion of 3 years therapy in 2 children with clinically stable Wilson's disease.The course as judged by clinical, biochemical and histological parameters was satisfactory in both patients. The urinary copper concentration reverted to <80 ug/24h (1.26 umol/24h) and the serum copper concentration decreased further during zinc sulphate therapy. In one child the rise in 24h urinary copper excretion observed after a challenge dose of D-P (20mg/kg) remained constant throughout the period of observation while the liver copper content fell from 1460 ug/g dry weight at diagnosis to 890 ug/g dry weight at reevaluation. In the other patient, however, the liver copper content as well as the 24h urinary copper excretion after D-P challenge increased during the third year of therapy.

We conclude that zinc sulphate is a low-toxic and well tolerated alternative for D-P. The quantity of zinc required to block copper absorption adequately, however depends greatly on not yet well identified individual factors. We recommend to restrict its use in patients who do not tolerate D-P well and suggest to monitor the treatment with a D-P challenge every 12 months and a liver biopsy if liver function deteriorates.

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The current treatment of Wilson's disease is to restrict copper intake and to promote the urinary excretion of this metal by the administration of an oral copper chelating agent, D-Penicillamine (1). Side effects and toxic reactions are frequently observed and in a few instances require to stop the therapy (2). Moreover non-compliance is nowadays the major problem of this life long treatment and might be partially due to a psychological aversion to the taste of this drug.

One may consider a different therapeutical approach; i.e. to inhibit the gastrointestinal absorption of copper by the administration of a copper binding agent. Substances such as carbacrylamide and potassium sulfide (3) however turned out to be unsuccessful at maintaining negative copper balance.

The antagonistic action of zinc on copper absorption was already known for years in veterinary medicine (4) when in 1961, Schouwink described the same effect in two patients with Wilson's disease. One of them has since then been treated succesfully during 25 years with zinc (5,6). The observation that large doses of zinc can inhibit copper absorption was further confirmed in sickle cell patients (7), and recently in a well controlled balance study of 5 patients with Wilson's disease (8). Zinc sulphate may thus be considered as a low-toxic and safe alternative to the classic therapy with D-Penicillamine in patients unable to tolerate the latter drug. We describe here the experience with two such children, treated for 3 years with zinc sulphate after an initial decoppering period with D-Penicillamine.

PATIENTS AND METHODS

The age of the children was 3 and 5 years when D-Penicillamine therapy was started and 4 and 8 years, respectively, when zinc sulphate was introduced. They were in stable clinical condition: Kayser-Fleischer rings, neurologic symptoms, jaundice and portal hypertension were absent. Patient 2 presented initially with a chronic active hepatitis like syndrome without splenomegaly, while patient 1 was an asymptomatic sibling of a patient known to have Wilson's disease. On admission both patients had elevated transaminases. Criteria for diagnosis were: liver copper level > 325 ug/g dry weight, urinary copper excretion > 80 ug/24h (1.26 umol/24h) before therapy, caeruloplasmin level < 20 mg/dl, abnormal radiocopper kinetics (no incorporation of 64 Cu in caeruloplasmin) and familial occurrence in patient 1.

Serum and 24 h urine for zinc and copper assays as well as liver biopsy specimens were collected in metal free containers. All metal analyses were performed in duplicate by atomic absorption spectrophotometry.The intravenous radiocopper test was performed according to a modification of a previously described protocol (9). ^{64}Cu (half life 12,7h) was used at a dosis of 0.03 mCi (6 millirem). Serum liver function tests were carried out at intervals of approximately three months.Liver biopsies were examined with transmission electron microscopy.

The zinc therapy regimen involved the administration of oral zinc sulphate with the meals, 3x150 mg/day initially, followed by a maintenance dose of 3x100 mg/day (Zn SO4.7H2O corresponding to 3 X 34 and 3 X 22,5 mg/day of elemental zinc, respectively). During challenge tests with D-Penicillamine (20 mg/kg), zinc therapy was discontinued 3 days before the challenge.

RESULTS

Figure 1 shows the course of serum copper values and 24 h urinary copper excretion throughout therapy in both patients. Initially serum copper fell after decoppering with D-Penicillamine to 28 ug/dl (4.4 umol/l) and 19 ug/dl (3 umol/l) respectively and decreased further during oral zinc sulphate therapy. 24h urinary copper excretion was elevated at diagnosis.It increased (as expected) during D-Penicillamine therapy, but after this treatment was discontinued, it returned to normal values for patients with liver diseases (<80 ug/24h or < 1.26 umol/24h) when the oral zinc sulphate regimen was reinstituted.

The liver function tests (e.g. transaminases), normalised after the initial decoppering period under D-Penicillamine and remained so during the first 3 years with zinc sulphate therapy. Table 1 shows the effect of a challenge dose of D-Penicillamine on 24 hr urinary copper excretion after 3, 15, 27 and 39 months of zinc therapy.

The disappearance rate of intravenously administered ^{64}Cu from the plasma in both patients measured at yearly intervals after the start of zinc therapy, showed little change and the curves obtained were consistent with the absence of copper accumulation.

Light microscopy showed initially a diffuse steatosis in both patients and a periportal inflammatory infiltrate with perilobular fibrosis and hepatocellular necrosis in patient 2. 3 Years after zinc sulphate therapy the liver histology was greatly improved and in both patients virtually normal. The ultrastructural changes (electron microscopy) were still characterised by the presence of heterogeneous multivesiculated inclusion bodies (lysosomes ?) in the hepatocytes. The mitochondria showed varied shapes and sizes with

deterioration of a substantial part of the cristae.
The liver copper content which was 350 ug/g dry weight after initial decoppering with D-Penicillamine in patient 2, rose to 1050 ug/g dry weight after 3 years of zinc sulfate therapy. In patient 1 the liver copper content decreased from 1460 ug/g dry weight at diagnosis to 890 ug/g dry weight at reevaluation.
No side-effects were observed.

DISCUSSION

There is now good evidence that long term zinc sulphate therapy can maintain neutral or negative copper balance in patients with clinically stable Wilson`s disease. Recently, Brewer et al (8) reported that pharmacological doses of oral zinc promote the faecal excretion of copper in D-Penicillamine treated Wilson's patients provided that the previously depleted total body zinc was restored. This view is supported by the normal liver function tests, by the reversion of the urinary copper excretion to normal, and by the maintenance of a low serum copper concentration during 3 years of zinc sulphate therapy in both children described here. The small but consistent rise in urinary copper excretion after a challenge dose of D-Penicillamine in patient 1 leads to the same conclusion. This is further confirmed by the course of the histological picture in both patients and by the determination of the liver copper content in patient 1.
The increase in liver copper content observed in patient 2, however, is disturbing and the absence of concomitant elevation of the transaminases values remains unexplained. This rise may be due to a sampling error with respect to the initial biopsy. In fact, such errors are not unusual in fibrotic tissue (10). On the other hand,

the dose of zinc sulphate may not have been large enough to prevent some reaccumulation of copper in the liver. That this could have occurred at the end of 3 years therapy in patient 2 can be deducted from the rise observed in 24 h urinary copper excretion after the challenge dose of D-Penicillamine. One could then speculate that zinc promotes the synthesis of metallothionein, not only in the intestinal - but also in the liver cells. Metallothionein is known to have a higher affinity for copper than for zinc (11). Any copper escaping the blocking effect of zinc on absorption will probably be bound to liver metallothionein and this mechanism may temporarily protect the tissue against the toxic effect of free copper, explaining the absence of symptoms observed.The quantity of zinc required to block the copper absorption adequately has thus to be adapted individually.

No previous data on liver copper content of Wilson's patients treated with zinc sulphate are known to us. Nevertheless, zinc sulphate has been shown since 1957 to be able to maintain a negative copper balance and a satisfactory clinical condition in several patients in the Netherlands (5). It can therefore be considered as a low-toxic, low-cost and safe alternative for D-Penicillamine provided the dosage is adjusted to individual needs. The effect of a low copper diet and of a specific zinc dose on the individual copper absorption are indeed still poorly understood at this moment. As long as zinc sulphate has not been proven to be effective in all patients, we suggest to prescribe it only if D-Penicillamine is either not tolerated or not available. We recommend to perform a challenge with a test dose of D-Penicillamine every 12 months and to adapt the maintenance dose of zinc sulphate to the outcome.

In case of doubt a liver biopsy is indicated.

As yet there is no clinical experience with zinc therapy in acutely ill patients with Wilson's disease.

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Figure 1: Serum copper and 24 h urinary copper excretion during D-Penicillamine and zinc sulphate therapy in patients 1 and 2 as a function of time.

Conversion-traditional units to SI: Copper 1 ug = 0.015 umol;

Zinc 1 ug =0.015 umol.

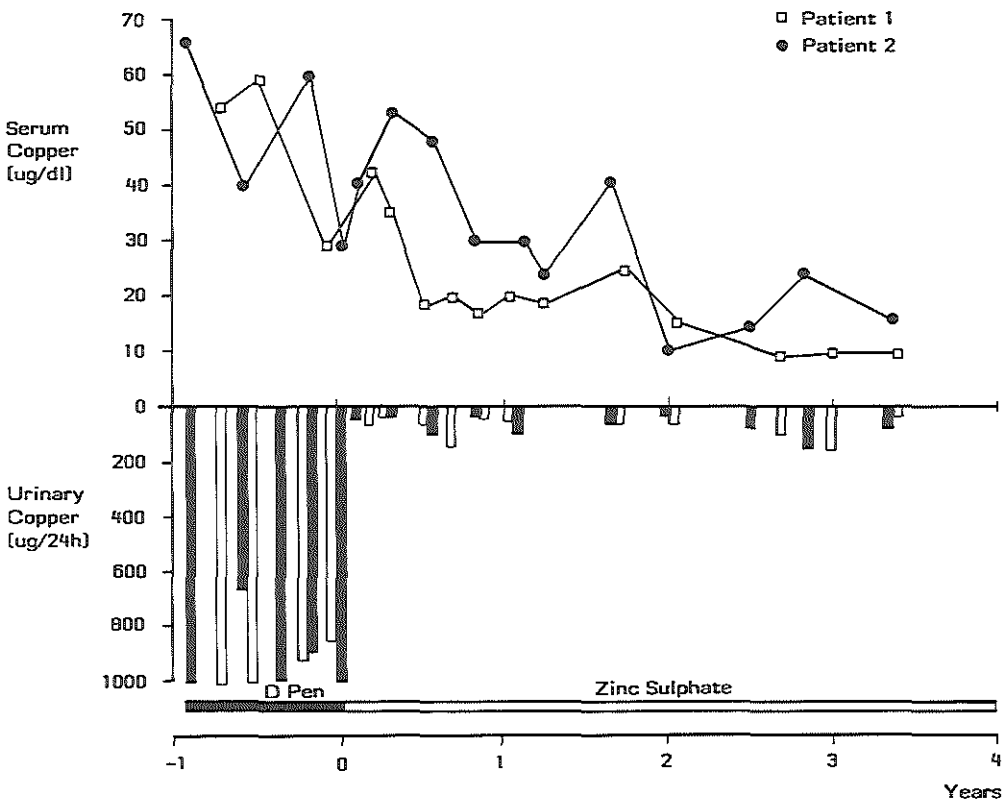

TABLE Urinary copper and zinc excretion after a test dose of D-Penicillamine

Time since start of treatment(months)	Patient 1		Patient 2	
	Zn(ug/24 h)	Cu(ug/24 h)	Zn(ug/24 h)	Cu(ug/24 h)
3	2060	250	1510	200
15	2200	340	1730	320
27	4400	200	1900	350
39	3150	210	3700	960

CHAPTER VI . FINAL DISCUSSION AND SUMMARY

The aim of the studies presented in this thesis was to investigate some pediatric aspects of trace element metabolism .The effects are described of 1) inadequate trace element intake , 2) trace element malabsorption ,3) urinary losses during therapy with D-Penicillamine and 4) zinc -copper interaction during therapy with zinc sulphate.Within this general framework our attention was especially focused on the trace element selenium, whose essentiality was virtually unrecognized at the beginning of these studies in 1978.Much attention was also paid to the different aspects of zinc and copper homeostasis in Wilson's disease, which represents one of nature's experiments of trace element interaction .Several questions,related to the treatment of this inherited metabolic disorder,had to be solved.

In the initial phase of the study ,it was decided to establish within our laboratory the levels of plasma selenium in healthy individuals of different ages . Hardly anything was known about selenium. Our studies confirmed and completed the scarce reports from New-Zealand and Germany ,that the plasma selenium level increases steadily. When expressed on a logarithmic scale,it rises almost linearly with age. The values found in infancy were significantly lower than the levels found in adults or in a group of older children, while younger children had intermediate levels.As to zinc and copper ,we found levels, in serum and plasma ,which were comparable (mean $\pm$ SD) to those previously reported in the literature . Unforeseen sources of metal contamination (as vacutainer rub-

ber stoppers) were looked for and eliminated . We saw the characteristic (physiological ?) decline in plasma zinc values at about three months of age in infants. It is thus mandatory, when one wishes to interpret a serum or plasma level of selenium, copper or zinc correctly, not only to define the age of the child but also to use appropriate reference values.It is also advisable to define time, fasting state and possible stress.

The nutritional aspects of selenium during infancy were further investigated.The importance of this aspect cannot be overemphazised, to quote Henry A.Schroeder[1] : "The trace elements are more important in nutrition than are the vitamins,in the sense that they cannot be synthesised by living matter, as is the case with vitamins.Thus they are the basic sparkplugs in the chemistry of life".

The selenium intake of healthy full term infants on breast milk, commercial formulas and nutritional support was evaluated.

It was found that the selenium content of breast milk is highest in colostrum, subsequently declines and finally levels off after one month. The daily intake of infants in Belgium was calculated to average 8 ug (3.4 - 9.1 ug). Still lower was the mean daily intake of infants fed adapted formulas (3.8 - 6.2 ug), while infants fed undiluted cow's milk would receive 8.7 to 12.4 ug/day.

The adequate and safe daily selenium intake for infants (10 to 40 ug/day) ,recommended by the U.S. Food and Nutrition Board, is thus not met in Belgium. This is reflected in the plasma levels, which are particularly low.These levels are not very far removed from the values found in children living in selenium deficient areas in China (mean $\pm$ SD : 1.7 $\pm$ 0.02 ug/100 ml).Here, the incidence of Keshan

disease is estimated at 1/200 children.At present, there is no clinical disorder ascribed to a marginal deficiency. How important indeed is an adequate selenium supply in human health care ? To quote Flohé [2] :" Subacute deficiency existing for some time might induce a metabolic or histopathological change, which in turn will trigger a disease at some future time,when the deficiency itself may not be detectable anymore." Although controversial, epidemiological studies with selenium supplementation in children with known selenium depletion and submitted to oxidative stress (premature infants and patients with cystic fibrosis) are urgently needed. More information on the toxicity of the various selenocompounds in man for this purpose is of utmost importance.

The infants on "total" (?) parenteral nutrition are virtually deprived of significant amounts of selenium ,even if they are regularly provided with fresh frozen plasma.The total amount given is insufficient to cover the requirement. The commercially available mineral solutions do not contain selenium at present.

Our study on infants on nutritional support showed a significant fall in plasma selenium value after only 4 weeks on parenteral nutrition .A parallel but delayed decrease in red blood cell glutathione peroxidase activity occured simultaneously. Noteworthy to say:in one infant the decline in selenium value occurred with a transient rise in transaminases. After reintroduction of enteral foods the selenium level increased progressively and reached reference values. This suggests that the selenium requirement was not met during nutritional support. We concluded that it is essential to provide longterm TPN patients with physiological amounts of selenium in order to prevent the progressive development of a deficiency

state.

The influence of steatorrhea, and thus malabsorption, upon the trace element requirements was evaluated in 13 children with cystic fibrosis .We found adequate zinc levels in 11 and a possible zinc deficiency in 2 patients.On the contrary ,the copper levels were high, as well as the urinary excretion of copper in some patients .The question has to be raised whether the biliary excretion of this metal is diminished in patients with cystic fibrosis and disturbed liver function. The plasma selenium levels were significantly reduced in comparison with an age-matched control group. Moreover we observed a negative correlation between the faecal fat excretion and the plasma selenium levels. Almost nothing is known about the mechanism of selenium absorption and this correlation was not described before. Since our publication in 1982, the presence of diminished selenium levels in children with cystic fibrosis, has been confirmed by others. The question of supplementation has not yet been answered.

Concluding ,we would like to underline the fact that each essential trace element has its own requirement, influenced by different metabolic pathways of excretion and absorption. For this reason we consider oral suppletion of trace element mixtures of fixed composition as generally inappropriate.

The problems of children with Wilson's disease are multiple. To evaluate the effect of the decoppering therapy in 4 children with Wilson's disease, we determined serial serum copper and zinc levels as well as 24 h urinary zinc and copper excretion during D-Penicillamine therapy and after a challenge dose of D-P. It was then

clear that the urinary copper excretion, after an initial rise, gradually declined during treatment as did the serum level. The urinary zinc excretion increased further. Zinc depletion was suspected in one patient in whom we documented the clinical features of zinc deficiency.Lowering the dose of D-P alleviated the symptoms as the urinary zinc loss was decreased by 50 %.The copper excretion remained unchanged. Our data showed clearly that D-P alters the metabolism of copper and zinc .This phenomenon is dose dependent and time related.We recommend that the dose of D-P during maintenance therapy should be adapted as to find an acceptable balance between copper accumulation and zinc depletion .

We also had the opportunity to investigate the effect of zinc-copper interaction for the treatment of Wilson's disease.It had already been shown in 1961 by Schouwink in Utrecht that pharmacological doses of zinc block the absorption of copper and thus prevent its accumulation in Wilson's disease. We evaluated the effect of oral zinc sulphate in 2 children with clinically stable Wilson's disease after completion of 3 years therapy .The clinical, biochemical and histological course was satisfactory in both patients.In one child the liver copper content fell.In the other child ,the liver copper content rose as did the 24 h copper excretion after a D-P challenge. We concluded that zinc sulphate is a well tolerated and low toxic alternative for D-P .The quantity of zinc required to block copper absorption adequately depends greatly on not yet well identified individual factors. Thus we recommend to restrict its use in patients who do not tolerate DP well .We suggested to monitor closely this treatment with a D-P challenge every 12 months and a liver biopsy if liver function deteriorates.

Having been involved with trace elements metabolism in childhood and infancy [3], we would like to conclude as Jonathan Shaw :" Perhaps the most remarkable fact about trace elements is not their scarcity but their abundance in the tissues of the human body."

When this work was started our knowledge of trace element requirements in infancy and childhood was not precise and still is . After years of neglect ,however, progress has been achieved very recently with a growing appreciation of the proven and potential importance of these micronutrients.In the human ,the premature infant and the child with a malabsorption syndrome appear to be at a particular risk to develop such deficiencies. This is a period when substantial increases in total body content of trace elements occur and an additional demand is made on the limited quantities available .The gastrointestinal tract has at the same time a limited capacity to absorb some of the trace elements,while the "very" adapted formulas provide protein , energy ,and other nutrients in abundance.

We are thus confronted with one of the paradoxes of this society : deficiency in the midst of opulence.

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SAMENVATTING

Dit proefschrift bevat de beschrijving van enkele aspekten van het spoorelement metabolisme bij de zuigeling en het oudere kind.
In hoofdstuk I worden de vragen die aan dit onderzoek ten grondslag liggen ingeleid,gevolgd door een kort overzicht van de literatuurgegevens over selenium ,koper en zink.Ingegaan wordt op de aanbevolen hoeveelheden van deze micronutrienten , evenals op de faktoren die deze hoeveelheden kunnen beinvloeden.
Hoofdstuk II bevat het onderzoek verricht naar de plasma selenium waarden en glutathion peroxidase aktiviteit in de rode bloedcellen als functie van de leeftijd. Bovendien wordt een beknopt overzicht gegeven van in de literatuur beschreven referentiewaarden van plasma koper en serum zink. Hieraan worden de eigen resultaten getoetst.
In hoofdstuk III wordt de seleniumconcentratie beschreven,aanwezig in moedermelk ,geadapteerde koemelk of semi-synthetische voeding . Tevens werd de concentratie spoorelementen in de gangbare oplossingen voor parenteraal gebruik bepaald.Het bleek dat in Belgie de gemiddelde hoeveelheid selenium die de zuigeling dagelijks via moedermelk krijgt, lager is dan in de meeste andere landen waarvoor gegevens bekend zijn. Dit geldt a fortiori voor geadapteerde koemelk en semi-synthetische voeding.Aansluitend werd het verloop van de selenium waarden in plasma en van de glutathion peroxidase aktiviteit bestudeerd in erythrocyten van zuigelingen, die langdurig parenteraal gevoed werden.

Hoofdstuk IV geeft de resultaten van een studie naar het metabolisme van selenium ,koper en zink bij 13 kinderen met pancreas fibrose . Bij 2 patienten werd een verlaagd serum zinkgehalte gevonden ,mogelijk duidend op een marginale deficientie .De overige resultaten vielen binnen de norm .Daarentegen waren de koperwaarden verhoogd. De plasma seleniumwaarden waren significant lager dan de seleniumspiegels van een qua leeftijd vergelijkbare controlegroep.
Uit de in dit hoofdstuk gerapporteerde bevindingen werd onder meer geconcludeerd dat kant en klare spoorelementen mengsels niet altijd geschikt zijn als suppletie therapie bij malabsorptie syndromen, omdat ieder element gekenmerkt is door eigen absorptie- en eliminatiewegen .
In hoofdstuk V.1 valt de nadruk op de zink en koper huishouding tijdens D-Penicillamine therapie bij 4 kinderen met Morbus Wilson. Het werd duidelijk dat D-Penicillamine niet alleen de urineuitscheiding van koper bevordert , maar ook in belangrijke mate de zinkuitscheiding beinvloedt .Dit effect is afhankelijk van de dosis, maar ook van de reeds bereikte uitdrijving van het gestapelde koper. Op klinische en biochemische gronden werd bij een patient een zinkdeficientie vermoed.
In hoofdstuk V.2 wordt ingegaan op de diverse aspecten van zinkkoper interaktie tijdens de behandeling van Morbus Wilson met zinksulfaat.Schouwink had reeds in 1961 in zijn proefschrift beschreven dat de koperabsorptie doeltreffend geremd wordt door pharmacologische doses zinksulfaat ,gegeven met de maaltijd .Men zou de accumulatie van koper in de lever en andere weefsels bij patienten met

de ziekte van Wilson aldus kunnen voorkomen.Het klinisch,biochemisch en histologisch verloop van beide in deze studie onderzochte patienten , was ,na drie jaar behandeling met zink ,bevredigend. Bij één patient daalde het zinkgehalte in de lever ,terwijl een stijging bij het tweede kind werd waargenomen .Uit deze resultaten werd geconcludeerd dat zinksulfaat een aanvaardbaar en weinig toxisch alternatief middel is voor D-Penicillamine.Voorlopig is deze behandeling alleen gerechtvaardigd bij patienten die D-P slecht verdragen . De hoeveelheid zink die noodzakelijk is om de absorptie van koper adequaat te remmen is nl. sterk afhankelijk van onvoldoend bekende ,per individu verschillende , faktoren.

Als algemene conclusie wordt in hoofstuk VI gesteld dat onze kennis over de behoeften aan spoorelementen van zuigelingen en kinderen vroeger weinig volledig was ,en ,ondanks grote vooruitgang , dit nog steeds is.

Tenslotte wens ik te eindigen met het motto van Jonathan Shaw : "Perhaps the most remarkable fact about trace elements is not their scarcity but their abundance in the tissues of the human body." (Amer.J.Dis.Child. 133:1260,1979.)

CURRICULUM VITAE

De schrijfster van dit proefschrift werd in 1946 te Antwerpen geboren.Twee jaar later ,verhuisde ze naar het toenmalig Belgisch Kongo om in 1953 naar Brussel terug te keren.Zij bezocht Het Heilig Hart Lyceum te Antwerpen en behaalde in I963 het diploma uitgereikt door de Centrale eksamenkommissie te Brussel .In hetzelfde jaar,begon zij de studie in de geneeskunde aan de Université Catholique de Louvain. In 1970,legde zij het artsexamen af.
Ze vertrok daarop naar Galveston,Texas,U.S.A. Aan de drie intensieve opleidingsjaren als "House Officer " aan de U.T.M.B., heeft ze een buitengewone herinnering behouden.Aan Dr. C.W.Daeschner dankt zij haar interesse in voeding en spoorelementen onderzoek, aan Dr.J.Simon haar opleiding in algemene pediatrie, aan Dr. G. Powell haar specialisatie in pediatrische gastroenterologie ,aan Dr. A. Goldman haar verbijf in het Shriners Hospital for Crippled Children .In 1973 , verhuisde ze naar Montreal om zich verder te specialiseren onder de leiding van Dr.C.Morin en Dr.C.Claude Roy. Dr.A.Weber ,model van een aktieve wetenschappelijke onderzoekster,is voor haar nog belangrijker geweest dan het behalen van het Certificate of the American Board of Pediatrics in 1975.
In 1975, startte ze haar huidige loopbaan onder de leiding en hoede van Dr.J. Fernandes in het Sophia Kinderziekenhuis te Rotterdam (Hoofd:Prof.H.K.A.Visser). Bij hem leerde ze de basismethodologie van het klinisch wetenschappelijk onderzoek en tevens de weg naar het laboratorium.Aan Dr.H.Degenhart dankt zij het woord "selenium".
In 1981 ,behaalde ze in Brussel het taaldiploma Nederlands,

Niveau 1 .Sindsdien werkt ze als seniorstaflid in het Algemeen Kinderziekenhuis Antwerpen (Hoofd : Prof.Dr.R.Clara).Ze is tevens consulente kindergastroenterologie aan het Akademisch Ziekenhuis te Antwerpen , en co-promotor van N.F.W.O. projekten aan de Universitaire Instelling Antwerpen (U.I.A.).