ZINC IN HUMAN NUTRITION

SUSAN J. FAIRWEATHER-TAIT

AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA

CONTENTS

INTRODUCTION

Interest in zinc nutrition was aroused 25 years ago when Zn deficiency, related primarily to diet, was shown to be the cause of dwarfism and hypogonadism among adolescents from the lowest social classes of Egypt and Iran (see Prasad, 1984). Since then a considerable amount of research has been carried out on Zn in order to understand its role in human nutrition. This review will attempt to summarize our present-day knowledge of Zn, concentrating primarily on the most active and exciting areas of research that have evolved over the past few years. Where possible, review articles will be cited to which the reader may refer to gain a fuller insight into a particular aspect of Zn nutrition. After a brief description of the biochemistry and metabolism of Zn, the most-recent evidence of problems in Zn nutrition will be presented. The reasons for their existence will be examined, together with a résumé of the various approaches taken to improve our understanding of Zn.

BIOCHEMISTRY AND METABOLISM OF ZN

The total amount of Zn in the human adult has been estimated to be approximately 2 g. More than 80% of this is found in bone, muscle, hair and skin, and a large number of enzymes require Zn for maximum catalytic activity, e.g., alcohol dehydrogenase (*EC* 1.1.1.1), RNA nucleotidyltransferases and alkaline phosphatase (*EC* 3.1.3.1). In addition, Zn may have a structural role, e.g. in superoxide dismutase (*EC* 1.15.1.1), and alcohol dehydrogenase, where it is bound to the apoprotein in fixed stoichiometric ratios. Zn plays a fundamental role in expression of the genetic potential; the synthesis, repair and structural integrity of nucleic acids require Zn. Therefore, it is not surprising that deficiency of Zn reduces growth in almost all biological systems via decreased cell replication. Initially this was attributed to the observation that DNA polymerase (*EC* 2.7.7.7) contains Zn as a functional component, but more-recent suggestions have been put forward implicating

other enzymes such as thymidine kinase (*EC* 2.7.1.21) (Cousins, 1986). The role of Zn in brain development and function has been reviewed by Sandstead (1985) and Wallwork (1987).

Zn is also involved in stabilizing membrane structures and in protection at the cellular level by preventing lipid peroxidation and reducing free-radical formation (Coppen *et al.* 1985). The latter function probably arises from the fact that the Zn–thiolate clusters in metallothionein are efficient at scavenging free hydroxyl radicals (Thornalley & Vasak, 1985).

Zn absorption has been reviewed by Kirchgessner & Weigand (1983) and Cousins (1985). Reports on the relative contribution of the different sections of the intestine vary, and it has been suggested that the major site of absorption might shift with differences in food matrix and body Zn status. Absorption can occur throughout the total length of the small intestine, but colonic absorption is limited (Sandstrom *et al.* 1986). Fractional Zn absorption falls with increasing dose (Istfan *et al.* 1983), but increases fairly rapidly in response to reductions in dietary Zn intake (Wada *et al.* 1985). Mechanisms of Zn absorption and homeostasis are a topic of debate and are based mainly on rat studies. Recent findings suggest that Zn uptake across the brush-border surface may occur partly by a regulated carrier-mediated diffusion mechanism, which responds homeostatically to the dietary Zn supply. Transport kinetics show evidence of both passive and saturable processes, but with Zn deficiency a greater portion of the total transport is via the saturable component. Above a certain lumen concentration transport may take place by passive diffusion (Menard & Cousins, 1983). The biphasic nature of Zn absorption is discussed by Solomons & Cousins (1984). The control of Zn homeostasis may operate via an increase in intestinal transport (Menard & Cousins, 1983) or a decrease in endogenous secretions of Zn into the intestine (Matseshe *et al.* 1980) and in sweat (Milne *et al.* 1983), or both. Jackson *et al.* (1984) have proposed that small daily variations in Zn intake are dealt with by alterations in gastrointestinal secretion of Zn, which is a rapidly responding mechanism, but that larger changes in Zn intake can only be adequately dealt with by changes in absorption. Although the latter is slower to take effect, it has a greater capacity to cope with large fluctuations in dietary Zn.

There are three stages in Zn absorption. First the Zn is chelated in the intestinal lumen by an endogenous factor before uptake at the brush border. The Zn is then transferred intracellularly by Zn-binding ligands (ZBL), such as low-molecular-weight ZBL, metallothionein (MT), and high-molecular-weight ZBL. Song (1987) suggests that the low-molecular-weight ZBL is a key regulator of intestinal Zn absorption, but the identity of this ligand is a matter of controversy. Seal & Heaton (1987) have isolated two proteins from rat mucosal cytosol that bind Zn. They concluded from their studies with ^{65}Zn that Zn entering the cytoplasm rapidly binds to a protein of molecular weight 6500 and is then actively transferred to a protein of molecular weight 45000. Finally, the Zn is removed from the basolateral membrane of the epithelial cells to enter the systemic circulation.

Approximately 65% of portal plasma Zn is associated with albumin, and the remainder with other proteins, notably α-2-macroglobulin and transferrin. Once absorbed, substantial amounts of Zn are taken up by the liver, then subsequently redistributed to other tissues, primarily bone and muscle. Since bone mineralization and reabsorption are controlled by the endocrine system which maintains calcium balance, Zn deposited in the mineralized matrix of bone cells is not readily mobilized. In contrast, a substantial amount of Zn may be supplied from the catabolism of muscle tissue for short-term redistribution to appropriate cellular sites. Hepatocytes are in dynamic equilibrium with the plasma Zn supply and contain two intracellular pools, the smaller of which is labile and may serve as an initial intermediate in Zn metabolism by hepatocytes as well as more general aspects of

Table 1. *Techniques used to measure zinc absorption*

Method	Advantages	Disadvantages
Metabolic balance	(a) Retention from complete diet can be measured	(a) Measures retention only
	(b) Generates useful information for estimating Zn requirements	(b) Small errors in intake or excretion lead to large errors in calculated retention
^{65}Zn and whole-body counting	(a) Assuming no problems with counting geometry, simple and accurate technique	(a) Correction required for endogenous ^{65}Zn losses
	(b) Can be used for single foods or meals	(b) Whole-body gamma counter required
		(c) Restricted application because of hazards of ionizing radiation
^{65}Zn balance	Can be used for single foods or meals	(a) Correction required for endogenous ^{65}Zn losses
		(b) Less accurate than whole-body counting
Stable-isotope balance	(a) Can be used safely in all human subjects	(a) Corrections required for naturally occurring stable isotope, and endogenous losses
	(b) Multi-isotope studies possible	(b) Measurement of stable isotopes more exacting than radioisotopes
	(c) Time constraints of radioisotope work not applicable	(c) Suitable mass spectrometer required
Plasma Zn tolerance curves	(a) Quick, easy method	(a) Qualitative
	(b) Expensive hardware not required	(b) Non-physiological doses required
		(c) Interpretation of results difficult

liver function related to Zn (Pattison & Cousins, 1986). Most of the Zn in the systemic circulation is localized in erythrocytes and leucocytes (80–90%), erythrocyte Zn but not leucocyte Zn being exchangeable with plasma Zn. Wastney *et al.* (1986) carried out a detailed analysis of the kinetics of Zn metabolism in adult subjects given oral or intravenous ^{65}Zn, and identified five major sites at which Zn metabolism is regulated, namely absorption in the gut, excretion in the urine, exchange with erythrocytes and muscle, and secretion into the gut.

TECHNIQUES USED TO MEASURE ZINC ABSORPTION

A number of different approaches have been used to study Zn absorption. These are summarized in Table 1, together with an indication of their major advantages or disadvantages.

Metabolic balance studies tend to underestimate absorption since a considerable portion of faecal Zn is endogenous, derived from pancreatic juice, bile and intestinal secretions (Matseshe *et al.* 1980), rather than unabsorbed dietary Zn.

Zn retention can be accurately measured using ^{65}Zn and a whole-body gamma counter (see Lykken, 1983), but does not usually equate with absorption because of the complexities of Zn metabolism. However, retention values can be corrected for endogenous excretion of absorbed radioactivity by a simple mathematical operation (Arvidsson *et al.* 1978). Payton *et al.* (1982) have developed an alternative method whereby Zn absorption can be measured from a single stool specimen collected 24–72 h after an oral dose of a ^{65}Zn-labelled Zn salt

ingested with a non-absorbed radioactive marker, namely ^{51}Cr. The two isotopes have similar intestinal transit times, thus allowing correction for endogenous ^{65}Zn losses, and the method has been successfully used to measure Zn absorption from turkey meat (Flanagan *et al.* 1985).

Interest in the use of enriched sources of stable isotopes of Zn to study Zn absorption has increased during the last few years. Three of the stable isotopes can be measured by neutron-activation analysis (Janghorbani & Young, 1982), but this method requires direct access to a neutron source plus pre- and post-irradiation sample clean-up to remove interfering isotopes. These difficulties have stimulated research into alternative methods using mass spectrometry. All the stable isotopes can be measured by mass spectrometry, e.g. thermal ionization (Turnland *et al.* 1984), fast atom bombardment (Fairweather-Tait *et al.* 1988; Peirce *et al.* 1987) and inductively-coupled plasma mass spectrometry (Date & Gray, 1983).

The plasma Zn-tolerance method has been criticised because the plasma Zn levels reflect the rate of oral Zn entering the circulation and removal of Zn from the plasma to the tissues. Molokhia *et al.* (1980) circumvented the problem of giving non-physiological doses of Zn by using the short-lived radioisotope ^{69m}Zn to calculate Zn absorption by deconvolution after oral and intravenously-administered doses.

EVIDENCE OF PROBLEMS ASSOCIATED WITH ZINC NUTRITION

It is apparent from the literature that certain groups of people are at risk with regard to Zn nutrition. There are a number of reasons for their vulnerability, including inadequate intake or low bioavailability, malabsorption, elevated losses (particularly associated with certain clinical conditions), or elevated requirements to support growth, pregnancy and lactation. The diagnosis of Zn deficiency is not easy as there is no single definitive test that can be used to assess Zn status (as discussed in detail, see p. 30). The case for Zn deficiency or the risk of it occurring, therefore, rests in many instances on circumstantial evidence, such as growth retardation which is reversed with Zn supplements, or low plasma Zn levels.

A number of clinical conditions predispose individuals to Zn deficiency. Clearly, the anorexia associated with some malignant illnesses results in a low intake of Zn, and the administration of total-parenteral-nutrition solutions not containing Zn will precipitate Zn-deficient states (Takagi *et al.* 1986). Anorexia nervosa leads to a low tissue Zn state, but is unlikely to cause overt Zn deficiency except in extreme cases (Ainley *et al.* 1986). Alcoholics and patients with liver disease generally exhibit hyperzincuria (Sullivan & Lankford, 1965), have low leucocyte Zn levels (Keeling *et al.* 1982), and a reduced absorptive capacity for Zn (Dinsmore *et al.* 1985). Preliminary findings suggest that patients with pancreatic insufficiency also have impaired Zn absorption (Watson *et al.* 1988). Renal patients have been shown to have reduced serum Zn concentrations, particularly with severe renal failure (Burge *et al.* 1984), but no explanation has been given for this observation. There is accumulating evidence that diabetes mellitus may lead to mild Zn deficiency (Mooradian & Morley, 1987), which is currently thought to be due to the combination of hyperzincuria and slightly impaired Zn absorption. Finally, the effect of trauma on Zn metabolism must be mentioned. Patients with burns or other skin disorders have a high dermal loss of Zn. In addition, any stress or trauma that causes loss of body protein, such as operative procedures, will at the same time result in loss of body Zn, mainly via the urine. At the same time there is a fall in serum Zn which is thought to reflect the movement of Zn to the liver and wound areas. The usefulness of therapeutic doses of Zn to maintain serum Zn levels is under debate since most research groups find that the fall

in serum Zn is not prevented even when the Zn is administered before the operation (Jiang *et al.* 1985).

Two genetic disorders have been linked to Zn deficiency. Acrodermatitis enteropathica, a recessive trait that is more commonly found amongst infants of Italian, Armenian or Iranian lineage, develops in the early months of life, and is fatal if untreated. The symptoms can be reversed with Zn supplementation, since the underlying cause of Zn deficiency is malabsorption. Patients with sickle-cell anaemia are often Zn deficient, probably due to an inadequate intake coupled with hyperzincuria.

Apart from people with genetic or clinical disorders, certain apparently-healthy members of the population may be susceptible to Zn deficiency. These include infants, growing children, and pregnant and lactating women, whose requirements are especially high in order to support growth. The elderly are also at risk, but for different reasons, namely low intakes of Zn and a reduced efficiency of absorption (Turnland *et al.* 1986). The latter observation may of course merely reflect a lower requirement for Zn by the elderly. However, Thomas *et al.* (1988) carried out duplicate diet analyses of long-stay hospital patients, aged 63–89 years, and found the mean Zn intake to be 5·5 mg d, which they concluded was low in comparison with levels of intake from healthy elderly people in metabolic equilibrium. The leucocyte Zn concentrations of long-stay patients were also low, particularly in those with leg ulcers or pressure sores, implying suboptimal Zn status.

Infants, especially those born prematurely, are susceptible to Zn deficiency (for review see Hambidge, 1986). Zn in human milk has a higher bioavailability than cow's milk, but infant formulas generally contain a much-higher level of Zn than that found in breast-milk in order to compensate for the lower absorption. Friel *et al.* (1985) examined nutrient intakes and growth in preterm and full-term infants, and found that Zn intake played a more-important role in explaining the height at 3 months and weight at 12 months than did any of the other measured variables. Walravens *et al.* (1988) found that infants aged 6–24 months with failure to thrive of primarily nutritional origin benefited from a daily 5 mg Zn supplement in terms of weight-for-age (males and females) and height-for-age (females only). Golden *et al.* (1985) demonstrated that inadequate Zn intake may be a limiting factor in the growth of severely malnourished infants, and Castillo-Duran *et al.* (1987) found that marasmic infants given Zn supplements (2 mg/d) had an enhanced weight gain during recovery which was unrelated to food intake. Zn also had a beneficial effect on infectious morbidity and host defence, a finding that has important clinical implications for the treatment of protein–energy malnutrition in developing countries.

Suboptimal Zn nutriture has been found in preschool children in Canada (Vanderkooy & Gibson, 1987). Boys with low hair Zn concentrations had a lower mean height-for-age percentile, and consumed less meat and fish but more Ca in their diet. Meat is considered to be one of the best sources of available Zn, and Ca may interfere with Zn absorption. Thus an inappropriate diet at this critical age may cause some problems associated with poor Zn nutrition. The second National Health and Nutrition Examination Survey (Pilch & Senti, 1985) found that 3·3% of 3–8-year-old boys had low serum Zn levels, and that this was the age-group most at risk amongst males. A greater percentage of girls aged 3–8 years had lower Zn levels than 9–19 year olds, although amongst females the age-range with the highest percentage of low serum Zn levels was 20–44 years. It appears that boys have a higher requirement for Zn than girls, possibly related to their faster growth rate. Zn supplements have been used with success in 7–13-year-old children in the USA of short stature with significantly-retarded bone age (Ghavami-Maibodi *et al.* 1983), when it was noted that hair Zn levels, which were initially low, increased during the course of supplementation. Unfortunately, no information regarding dietary Zn intakes was given.

There is considerable interest in the role of Zn in reproduction (Apgar, 1985). Since the drastic effects of severe maternal Zn deficiency on the rat fetus were demonstrated (Hurley, 1985), attempts have been made to assess the effect of suboptimal Zn nutriture on fetal development in humans. Although it is extremely unlikely that pregnant women ever undergo Zn depletion as severe as that used in the earlier studies on laboratory animals, several studies support the hypothesis that low birth weight is in some way linked to poor Zn nutrition during pregnancy (Simmer & Thompson, 1986).

Intrauterine growth retardation has been linked to low polymorphonuclear (PMN) Zn concentrations, and 85% of mothers having small-for-gestational-age infants were selected from low maternal PMN Zn levels or smoking during pregnancy, or both (Simmer & Thompson, 1985). When nutrient intake during the last trimester of pregnancy was assessed by dietary recall in mothers shortly after birth, low Zn intake was found to be significantly associated with intrauterine growth retardation (Simmer *et al.* 1987*a*). Maternal plasma Zn levels have been shown to be negatively correlated with birth weight in vegetarian women (Abu-Assal & Craig, 1984) and also women in Australia (McMichael *et al.* 1982), but the validity of assessing Zn status by means of plasma Zn levels in pregnancy is questionable. Ward *et al.* (1987) analysed placentas from 100 women who had obstetrically normal births and found that placental Zn concentrations were positively correlated with birth weight and head circumference over the lower end of the 'normal' range.

Oral Zn supplements (20 mg/d from the 12th week onwards) have been shown to reduce the overall complication rate for both mother and fetus, in particular small- or large-for-gestational-age infants (Kynast & Saling, 1986). However, by 12 weeks the development of most of the fetal tissues and organs is complete and subsequent Zn deprivation will have an adverse effect on fetal growth rather than a teratogenic effect.

The effect of maternal Zn status on pregnancy outcome obviously depends on the degree of deficiency, since a number of investigators have failed to show any link between Zn nutrition and birthweight. Hunt *et al.* (1985) supplemented low-income teenagers of Mexican descent (mean dietary intake of 10 mg Zn/d) with 20 mg Zn daily throughout pregnancy, but Zn supplementation did not affect the outcome of pregnancy compared with controls. A similar study was performed by Hambidge *et al.* (1983) who gave a supplement of 11 mg Zn/d but found no effect on pregnancy outcome or birth weight. Tuttle *et al.* (1985) found no association between intravascular mass of Zn and percentile-birth-weight distribution in women taking in 9 mg Zn/d, and Campbell-Brown *et al.* (1985) were unable to demonstrate any association between birth weight and Zn status in pregnant Hindu Asians and indigenous Europeans.

Many studies show that women do not increase their Zn intakes during pregnancy, yet the demand for absorbed Zn increases by up to 0·6 mg/d during late gestation (Swanson & King, 1987). Results from rat studies suggest that there is an adaptive response to meet the increased demands of pregnancy in terms of increased absorption (Fairweather-Tait *et al.* 1984), but there appears to be no conservation of body Zn by means of reduced Zn turnover (Fairweather-Tait *et al.* 1985). In humans, no evidence for adaptive changes has yet been described and Swanson *et al.* (1983) found no effect of diet or pregnancy on ^{70}Zn absorption.

The observed decline in circulating Zn in pregnancy is normal and reflects maternal–fetal transfer of Zn and expansion of maternal plasma volume. Zimmerman *et al.* (1984) contend that each of the major transport proteins for Zn has a specific role in Zn homeostasis, and that the pool of loosely bound Zn carried by albumin and amino acids (principally histidine and cysteine) is likely to provide rapid exchange of Zn to the placenta and fetus. Thus total plasma Zn levels are probably not a very useful measure of Zn metabolism in pregnancy.

Another area of concern is the effect of iron supplementation in pregnancy on Zn status. Meadows *et al.* (1983) demonstrated a fall in Zn absorption as a result of previous

administration of Fe. The fact that the Fe and Zn were not administered together led the authors to suggest that the Fe was interfering with Zn absorption at the level of the intestinal mucosa rather than in the lumen, but the doses of Zn used for the absorption test were well above dietary levels. More recently, this research group has confirmed their original findings using smaller doses of Zn (25 mg), and at the same time have shown that folate also interferes with Zn absorption (Simmer *et al.* 1987*b*). However, they used plasma Zn changes to give a quantitative estimate of absorption, which is not the method of choice. Sheldon *et al.* (1985) found that Fe supplements did not influence the changes in serum Zn levels seen in healthy or insulin-dependent diabetic mothers, but it is not known to what extent serum Zn represents metabolically-active Zn.

Following birth, maternal plasma Zn concentrations gradually return to pre-conception values, but in lactating women they are still lower than non-pregnant controls 9 weeks after birth (Qvist *et al.* 1986). Clearly, lactation imposes a drain on maternal Zn, but the fact that breast-milk Zn concentrations progressively fall over time may reflect a homeostatic mechanism for conserving maternal Zn (Krebs & Hambidge, 1986).

BIOAVAILABILITY OF ZINC

Zn is not fully absorbed from the diet, the actual amount being dependent on a number of dietary and physiological variables. Estimates of bioavailability are generally made from studies of absorption and retention, and a comprehensive review on this topic has been published recently (Solomons, 1982). The reader is also referred to a symposium sponsored by the American Chemical Society which was held on nutritional bioavailability of Zn in 1982 (Inglett, 1983).

The physicochemical form of Zn in a food is an important determinant of its bioavailability, but may be significantly modified by certain dietary constituents, as indicated in Table 2. Most substances reduce Zn availability by combining with soluble Zn in the intestinal lumen to form an unabsorbable complex. The most-potent inhibitor of Zn absorption of practical significance is probably phytic acid (*myo*-inositol hexaphosphate), found in most cereal grains and seed legumes.

A number of studies have shown that wheat bran reduces Zn absorption (e.g. Farah *et al.* 1984), which is thought to be primarily due to its high phytate content, since Zn absorption increases when the phytate content of bread is reduced by fermentation (Navert *et al.* 1985). The effect of phytate on Zn absorption largely depends on the phytate:Zn molar ratio as illustrated by the work of Turnland *et al.* (1984). Although Ca *per se* probably does not affect Zn bioavailability (Spencer *et al.* 1983), Ca is important in the presence of phytate since Ca–Zn–phytate complex is more insoluble than that formed by either element when combined separately with phytate. Ellis *et al.* (1987) have suggested that the critical values of phytate:Zn and phytate × Ca:Zn molar ratios are > 10 and > 200 respectively. Most omnivorous diets have ratios well below these, but certain diets may exceed this threshold, notably lacto-ovo vegetarian diets. Foods which may make a significant contribution towards reaching these critical ratios include unleavened wholemeal chapatti bread, tanok, untoasted muesli, soya-bean flour, health-food snack bars, and dairy products (Bindra *et al.* 1986; McKenzie-Parnell & Guthrie, 1986). Since meat is an important source of Zn in the diet, the substitution of meat with soya-bean products, usually high in phytate, may create problems with Zn nutrition, but this depends on the degree of replacement (Sandstrom *et al.* 1987*b*), and the composition of the rest of the diet. Cossack & Prasad (1983) concluded from their studies that 15 mg dietary Zn may not be sufficient to meet the daily requirement for adults if soya-bean protein is the major source of protein.

Other dietary constituents that significantly reduce Zn availability include oxalic acid,

Table 2. *Dietary factors affecting zinc bioavailability*

Inhibitors	Enhancers
Phytate (plus calcium)	Congeners in red wine
High-fibre foods, especially bran	Histidine
Hemicellulose	Animal protein
Oxalate	
Orange juice (ascorbic acid?)	
Iron	
Tin	
Maillard reaction products	

but probably only in the presence of dietary fibre (Kelsay, 1983), hemicellulose (Drews *et al.* 1979), orange juice, (active constituent ascorbic acid?) (Flanagan *et al.* 1985), and other inorganic elements that compete with Zn for absorption, e.g. Fe (Solomons & Jacobs, 1981), and tin (Solomons *et al.* 1983). Processing of food can also affect Zn bioavailability. The observation that Zn absorption was lower from browned cornflakes than the untoasted product was thought to be due to the presence of Maillard reaction products (Lykken *et al.* 1986). Extrusion cooking of a high-fibre cereal product reduced Zn availability in ileostomy subjects (Kivisto *et al.* 1986), which was attributed to the deactivation of bran phytase (*EC* 3.1.3.26) on extrusion cooking, but not in normal adults (Fairweather-Tait *et al.* 1988). The different results obtained in the latter two studies are probably due to chemical differences in the cereal products or physiological differences between the two groups of subjects.

A few substances have been shown to increase Zn availability, and these include the congeners of red wine (McDonald & Margen, 1980), the amino acid histidine (Scholmerich *et al.* 1987), and animal proteins such as those present in milk and cheese (Frohlich & Sandstrom, 1983).

DIAGNOSIS OF ZINC DEFICIENCY AND ASSESSMENT OF STATUS

There is a wide spectrum of clinical manifestations of Zn deficiency, depending on the degree of severity (Prasad, 1985). Laboratory diagnosis of severe deficiency is relatively simple, but marginal Zn deficiency is extremely difficult to confirm due to the lack of suitable methods of assessing Zn status. Various attempts have been made to develop a sensitive and reliable measure of Zn status as summarized in Table 3, but as yet there is no single method available. At present, the best indication of Zn deficiency is the biochemical and clinical response made to Zn supplements, but this is essentially retrospective. The level of Zn in plasma (or serum) does not always reflect body Zn status. Apart from diurnal fluctuations, there are other conditions, unrelated to Zn nutrition, that cause changes in plasma levels, e.g. the fall associated with infection, and with the use of oral contraceptives (King, 1987). Therefore, plasma Zn concentrations must be interpreted cautiously. Leucocyte Zn is a very-useful indicator of Zn status (Patrick & Dervish, 1984), but requires technical skill and a fairly large volume of blood. Bunker *et al.* (1984) have published values for leucocyte Zn in a group of elderly people which should serve as a useful reference standard. Serum alkaline phosphatase is low in Zn deficiency and rises following Zn therapy, indicating that serial determinations may be a useful aid in diagnosing Zn deficiency (Weismann & Hoyer, 1985; Baer *et al.* 1985). An alternative approach to the

Table 3. *Some of the techniques used to assess zinc status*

Useful indices
Biochemical and clinical response to Zn supplements
Plasma or serum Zn
Leucocyte Zn
Plasma or serum alkaline phosphatase (*EC* 3.1.3.1)
Erythrocyte metallothionein
Urinary metallothionein
Less-reliable measures
Erythrocyte Zn
Hair Zn
Salivary Zn
Fingernail Zn
Urinary Zn
Sweat Zn
Erythrocyte carbonic anhydrase (*EC* 4.2.1.1)
Platelet aggregation
Zn tolerance tests
Taste acuity
Dark adaptation

problem of assessing Zn status lies in the recent development of a radioimmunoassay to measure metallothionein in rats (Bremner *et al.* 1987*a*). Metallothionein is an important metal-binding protein that occurs in varying amounts in a wide range of tissues (Bremner, 1987*a*) its concentration being affected by exposure to many elements. The biological function of metallothionein is still a matter of considerable conjecture (Bremner, 1987*b*), but experiments to date have shown that the concentration of metallothionein in the blood and urine of rats appears to reflect body Zn status, unaffected by food restriction or cold stress (Bremner *et al.* 1987*b*). It may, therefore, be a useful aid in the diagnosis of Zn deficiency, once suitable radioimmunoassays are available for human studies.

The usefulness of other measures of Zn status is open to some debate (see Solomons, 1979). Medeiros *et al.* (1987) failed to show any effect of Zn supplementation on hair Zn levels in men of adequate Zn status, thereby eliminating hair Zn as an indicator of status in non-Zn-deficient subjects. Hair Zn concentrations depend not only on the delivery of Zn to the root, but also on the rate of hair growth, and Zn deficiency itself may impair the growth of hair, and actually result in increased Zn concentrations. Platelet aggregation has been shown to be impaired when plasma Zn levels are low following Zn deprivation, but is restored to normal within 19 h of Zn supplementation (Gordon *et al.* 1982). Further work is required to assess the importance of this finding. Zn tolerance tests (Fickel *et al.* 1986), taste acuity (Bales *et al.* 1986), and dark adaptation (Sandstrom *et al.* 1987*a*) are not good measures of Zn status.

REQUIREMENTS FOR ZINC

Human Zn requirements and recommended dietary allowances are controversial issues. The USA is one of the few countries at present that publishes official values for Zn (National Academy of Sciences, 1980) (see Table 4) and these are currently under review. Requirements for Zn are assessed by means of balance studies, from measurements of tissue endogenous losses, and from the functional response to a marginal Zn intake. Recommended allowances are then calculated from the estimated requirements, to be adequate to meet the known nutritional needs of practically all healthy persons. It is

Table 4. *Recommended daily dietary allowances for zinc (mg)*

	(US) NAS (1980)		WHO (1973)			
				Bioavailability of Zn in the diet (%)		
Category	Age (years)	Zn	Age (years)	10	20	40
Infants	0·0–0·5	3	0·0–0·3	12·5	6·3	3·1
	0·5–1·0	5	0·4–1·0	11·0	5·5	2·8
Boys	1–10	10	1–10	16·0	8·0	4·0
Girls	1–10	10	1–9	15·5	7·8	3·9
Males	11–14	15	11–17	28·0	14·0	7·0
	15–18	18	18+	22·0	11·0	5·5
	19+	15				
Females	11+	15	10–13	26·5	13·3	6·6
			14+	22·0	11·0	5·5
Pregnant	—	20	0–20 week	25·5	12·8	6·4
			20–30 week	29·0	14·5	7·3
			30–40 week	30·0	15·0	7·5
Lactating	—	25	—	54·5	27·3	13·7

NAS, National Academy of Sciences; WHO, World Health Organization.

important to remember that they are set at levels sufficiently above the average physiological requirement to just exceed the upper range of needs of almost-all individuals of a given age and sex category, i.e. two standard deviations above the mean value of requirements, and encompassing all but 2·5% of the range of individual requirements.

Regarding the question of bioavailability, an expert committee set up by the World Health Organization (1973) suggested three levels of Zn intake for each age-group, depending on the bioavailability of Zn in the diet. Thus, they categorized diets as containing Zn of 10, 20 or 40% bioavailability. The highest value is roughly equivalent to a Western diet, and the lowest value is that found in a diet containing substantial amounts of inhibitors, such as phytate from wheat bran, plus low intakes of meat. This approach serves to emphasize the importance of bioavailability and illustrates how dietary bioavailability may be the predisposing factor in determining whether or not the Zn intake of a group of people satisfies their requirements.

King (1986) presents preliminary evidence to suggest that tissue Zn status influences endogenous losses, and therefore the dietary need. Thus individuals in good status may require higher amounts of Zn in their diets than individuals in poor status. In addition, there is clear evidence that man can adapt to reductions in Zn intake by reducing urinary and faecal excretion. There are many dietary studies in which the mean Zn intake of an apparently healthy group of subjects is well below the recommended level (Record *et al.* 1985; Thomas *et al.* 1986). The high intakes recommended for pregnancy appear to present a particular problem, and it is unlikely that they can be achieved without dietary supplementation of the whole female population (Taper *et al.* 1985), an option that has been rejected by Hytten (1985). Allowances for lactation are even higher. Krebs & Hambidge (1986) found that women receiving 25 mg Zn/d had a slower rate of decline in milk zinc concentration than those receiving 11 mg/d but only after 6 months, which is the

time by which most infants are weaned. In fact, Ruz (1984) contends that it is impossible for infants up to 6 months to achieve the recommended level of 3 mg/d from breast-milk alone. It will not be possible to settle the arguments surrounding Zn requirements until there are reliable measures of Zn status that can be routinely used. Clearly, a great deal more research is needed in order improve our understanding of the role of Zn in human nutrition.

REFERENCES

Abu-Assal, M. J. & Craig, W. J. (1984). The zinc status of pregnant vegetarian women. *Nutrition Reports International* **29**, 485–494.

Ainley, C. C., Cason, J., Carlsson, L., Thompson, R. P. H., Slavin, B. M. & Norton, K. R. W. (1986). Zinc state in anorexia nervosa. *British Medical Journal* **293**, 992–993.

Apgar, J. (1985). Zinc and reproduction. *Annual Review of Nutrition* **5**, 43–68.

Arvidsson, B., Cederblad, A., Bjorn-Rasmussen, E. & Sandstrom, B. (1978). A radionuclide technique for studies of zinc absorption in man. *International Journal of Nuclear Medicine and Biology* **5**, 104–109.

Baer, M. T., King, J. C., Tamura, T., Margen, S., Bradfield, R. B., Weston, W. L. & Daugherty, N. A. (1985). Nitrogen utilization, enzyme activity, glucose intolerance and leukocyte chemotaxis in human experimental zinc depletion. *American Journal of Clinical Nutrition* **41**, 1220–1235.

Bales, C. W., Steinman, L. C., Freeland-Graves, J. H., Stone, J. M. & Young, R. K. (1986). The effect of age on plasma zinc uptake and taste acuity. *American Journal of Clinical Nutrition* **44**, 664–669.

Bindra, G. S., Gibson, R. S. & Thompson, L. U. (1986). [Phytate][calcium]/[zinc] ratios in asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. *Nutrition Research* **6**, 475–483.

Bremner, I. (1987*a*). Interactions between metallothionein and trace elements. *Progress in Food and Nutrition Science* **11**, 1–37.

Bremner, I. (1987*b*). Nutritional and physiological significance of metallothionein. *Experientia, Supplementum* **52**, 81–107.

Bremner, I., Mehra, R. K. & Sato, M. (1987*a*). Metallothionein in blood, bile and urine. *Experientia, Supplementum* **52**, 507–517.

Bremner, I., Morrison, J. N., Wood, A. M. & Arthur, J. R. (1987*b*). Effects of changes in dietary zinc, copper and selenium supply and of endotoxin administration on metallothionein I concentrations in blood cells and urine in the rat. *Journal of Nutrition* **117**, 1595–1602.

Bunker, V. W., Hinks, L. J., Lawson, M. S. & Clayton, B. E. (1984). Assessment of zinc and copper status of healthy elderly people using metabolic balance studies and measurement of leucocyte concentrations. *American Journal of Clinical Nutrition* **40**, 1096–1102.

Burge, J. C., Schemmel, R. A., Park, H. S. & Greene, J. A. III (1984). Taste acuity and zinc status in chronic renal disease. *Journal of the American Dietetic Association* **84**, 1203–1206.

Campbell-Brown, M., Ward, R. J., Haines, A. P., North, W. R. S., Abraham, R., McFadyen, I. R., Turnland, J. R. & King, J. C. (1985). Zinc and copper in Asian pregnancies – is there evidence for a nutritional deficiency? *British Journal of Obstetrics and Gynaecology* **92**, 875–885.

Castillo-Duran, C., Heresi, G., Fisberg, M. & Vauy, R. (1987). Controlled trial of zinc supplementation during recovery from malnutrition: effects on growth and immune function. *American Journal of Clinical Nutrition* **45**, 602–608.

Coppen, D. E., Cousins, R. J. & Richardson, D. E. (1985). Effect of zinc on chemically induced peroxidation in rat liver parenchymal cells in primary culture. *Federation Proceedings* **44**, 6404.

Cossack, Z. T. & Prasad, A. S. (1983). Effect of protein source on the bioavailability of zinc in human subjects. *Nutrition Research* **3**, 23–31.

Cousins, R. J. (1985). Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiological Reviews* **65**, 238–309.

Cousins, R. J. (1986). Towards a molecular understanding of zinc metabolism. *Clinical Physiology and Biochemistry* **4**, 20–30.

Date, A. R. & Gray, A. L. (1983). Isotope ratio measurements in solution samples using a plasma ion source. *International Journal of Mass Spectrometry and Ion Physics* **48**, 357–360.

Dinsmore, W. W., Callender, M. E., McMaster, D. & Love, A. H. G. (1985). The absorption of zinc from a standardized meal in alcoholic and in normal volunteers. *American Journal of Clinical Nutrition* **42**, 688–693.

Drews, L. M., Kies, C. & Fox, H. M. (1979). Effect of dietary fiber on copper, zinc and magnesium utilization by adolescent boys. *American Journal of Clinical Nutrition* **32**, 1893–1898.

Ellis, R., Kelsay, J. L., Reynolds, R. D., Morris, E. R., Moser, P. B. & Frazier, C. W. (1987). Phytate:zinc and phytate × calcium:zinc millimolar ratios in self-selected diets of Americans, Asian Indians, and Nepalese. *Journal of the American Dietetic Association* **87**, 1043–1047.

Fairweather-Tait, S. J., Portwood, D. E., Symss, L. L., Eagles, J. & Minski, M. J. (1988). Iron and zinc absorption in human subjects from a mixed meal of extruded and non-extruded wheat bran and flour. *American Journal of Clinical Nutrition*. (In the Press).

Fairweather-Tait, S. J., Wright, A. J. A., Cooke, J. & Franklin, J. (1985). Studies of zinc metabolism in pregnant and lactating rats. *British Journal of Nutrition* **54**, 401–413.

Fairweather-Tait, S. J., Wright, A. J. A. & Williams, C. M. (1984). Zinc metabolism in pregnant and lactating rats and the effect of varying iron:Zn in the diet. *British Journal of Nutrition* **52**, 205–213.

Farah, D. A., Hall, M. J., Mills, P. R. & Russell, R. I. (1984). Effect of wheat bran on zinc absorption. *Human Nutrition: Clinical Nutrition* **38C**, 433–441.

Fickel, J. J., Freeland-Graves, J. H. & Roby, M. J. (1986). Zinc tolerance tests in zinc deficient and zinc supplemented diets. *American Journal of Clinical Nutrition* **43**, 47–58.

Flanagan, P. R., Cluett, J., Chamberlain, M. J. & Valberg, L. S. (1985). Dual-isotope method for determination of human zinc absorption: the use of a test meal of turkey meat. *Journal of Nutrition* **115**, 111–122.

Friel, J. K., Gibson, R. S., Kawash, G. F. & Watts, J. (1985). Dietary zinc intake and growth during infancy. *Journal of Pediatric Gastroenterology and Nutrition* **4**, 746–751.

Frohlich, W. & Sandstrom, B. (1983). Zinc absorption from composite meals. In *Nutritional Bioavailability of Zinc, American Chemical Society Symposium Series* no. 210, pp. 211–221 [G. E. Inglett, editor]. Washington, DC: American Chemical Society.

Ghavami-Maibodi, S. Z., Collipp, P. J., Castro-Magana, M., Stewart, C. & Chen, S. Y. (1983). Effect of oral zinc supplements on growth, hormonal levels, and zinc in healthy short children. *Annals of Nutrition and Metabolism* **27**, 214–219.

Golden, M. H. N., Golden, B. E. & Bennett, F. I. (1985). Relationship of trace element deficiencies to malnutrition. In *Trace Elements in Nutrition of Children*, pp. 185–207 [R. K. Chandra, editor]. New York: Raven Press.

Gordon, P. R., Woodruff, C. W., Anderson, H. L. & O'Dell, B. L. (1982). Effect of acute zinc deprivation on plasma zinc and platelet aggregation in adult males. *American Journal of Clinical Nutrition* **35**, 113–119.

Hambidge, K. M. (1986). Zinc deficiency in the weanling – how important? *Acta Paediatrica Scandinavica*, Supplement **323**, 52–58.

Hambidge, K. M., Krebs, N. F., Jacobs, M. A., Favier, A., Guyette, L. & Ikle, D. N. (1983). Zinc nutritional status during pregnancy: a longitudinal study. *American Journal of Clinical Nutrition* **37**, 429–442.

Hunt, I. F., Murphy, N. J., Cleaver, A. E., Faraji, B., Swendseid, M. E., Browdy, B. L., Coulson, A. H., Clark, V. A., Settlage, R. H. & Smith, J. C. Jr (1985). Zinc supplementation during pregnancy in low-income teenagers of Mexican descent: effects on selected blood constituents and on progress and outcome of pregnancy. *American Journal of Clinical Nutrition* **42**, 815–828.

Hurley, L. S. (1985). Trace elements in prenatal and neonatal development: zinc and manganese. In *Trace Elements in Nutrition of Children*, pp. 121–135 [R. K. Chandra, editor]. New York: Raven Press.

Hytten, F. E. (1985). Do pregnant women need zinc supplements? *British Journal of Obstetrics and Gynaecology* **92**, 873–874.

Inglett, G. E. (ed.) (1983). *Nutritional Bioavailability of Zinc. American Chemical Society Symposium Series* no. 210. Washington, DC: American Chemical Society.

Istfan, N. W., Janghorbani, M. & Young, V. R. (1983). Absorption of stable ^{70}Zn in healthy young men in relation to zinc intake. *American Journal of Clinical Nutrition* **38**, 187–194.

Jackson, M. J., Jones, D. A., Edwards, R. H. T., Swainbank, I. G. & Coleman, M. L. (1984). Zinc homeostasis in man: studies using a new stable isotope-dilution technique. *British Journal of Nutrition* **51**, 199–208.

Janghorbani, M. & Young, V. R. (1982). Stable isotopes in studies of dietary mineral bioavailability in humans with special reference to zinc. In *Clinical, Biochemical, and Nutritional Aspects of Trace Elements*, pp. 447–468 [A. S. Prasad, editor]. New York: Alan R. Liss.

Jiang, A., Yang, N., Jiao, K., Zhu, Y., Fei, L. & Tseng, H. (1985). Postoperative fall in serum zinc concentrations unaffected by intravenous zinc therapy. *Journal of Parenteral and Enteral Nutrition* **9**, 196–198.

Keeling, P. W. N., O'Day, J., Ruse, W. & Thompson, R. P. H. (1982). Zinc deficiency and photoreceptor dysfunction in chronic liver disease. *Clinical Science* **62**, 109–111.

Kelsay, J. L. (1983). Effect of fiber and oxalic acid on zinc balance of adult humans. In *Nutritional Bioavailability of Zinc. American Chemical Society Symposium Series* no. 210, pp. 127–143 [G. E. Inglett, editor]. Washington, DC: American Chemical Society.

King, J. C. (1986). Assessment of techniques for determining human zinc requirements. *Journal of the American Dietetic Association* **86**, 1523–1528.

King, J. C. (1987). Do women using oral contraceptive agents require extra zinc? *Journal of Nutrition* **117**, 217–219.

Kirchgessner, M. & Weigand, E. (1983). Zinc absorption and excretion in relation to nutrition. In *Metal Ions in Biological Systems*, pp. 319–361 [H. Sigel, editor]. New York: Marcel Dekker.

Kivisto, B., Andersson, H., Cederblad, G., Sandberg, A.-S. & Sandstrom, B. (1986). Extrusion cooking of a high-fibre cereal product. *British Journal of Nutrition* **55**, 255–260.

Krebs, N. F. & Hambidge, K. M. (1986). Zinc requirements and zinc intakes of breast-fed infants. *American Journal of Clinical Nutrition* **43**, 288–292.

Kynast, G. & Saling, E. (1986). Effect of oral zinc application during pregnancy. *Gynecologic and Obstetric Investigation* **21**, 117–123.
Lykken, G. I. (1983). A whole body counting technique using ultralow doses of ^{59}Fe and ^{65}Zn in absorption and retention studies in humans. *American Journal of Clinical Nutrition* **37**, 652–662.
Lykken, G. I., Mahalko, J., Johnson, P. E., Milne, D., Sandstead, H. H., Garcia, W. J., Dintzis, F. R. & Inglett, G. E. (1986). Effect of browned and unbrowned corn products intrinsically labelled with ^{65}Zn on absorption of ^{65}Zn in humans. *Journal of Nutrition* **116**, 795–801.
McDonald, J. T. & Margen, S. (1980). Wine versus ethanol in human nutrition. IV. Zinc balance. *American Journal of Clinical Nutrition* **33**, 1096–1102.
McKenzie-Parnell, J. M. & Guthrie, B. E. (1986). The phytate and mineral content of some cereals, cereal products, legumes, legume products, snack bars, and nuts available in New Zealand. *Biological Trace Element Research* **10**, 107–121.
McMichael, A. J., Dreosti, I. E., Gibson, G. T., Hartshorne, J. M., Buckley, R. A. & Colley, D. P. (1982). A prospective study of serial maternal serum zinc levels and pregnancy outcome. *Early Human Development* **7**, 59–69.
Matseshe, J. W., Phillips, S. F., Malagelada, J.-R. & McCall, J. T. (1980). Recovery of dietary iron and zinc from the proximal intestine of healthy man: studies of different meals and supplements. *American Journal of Clinical Nutrition* **33**, 1946–1953.
Meadows, N. J., Grainger, S. L., Ruse, W., Keeling, P. W. N. & Thompson, R. P. H. (1983). Oral iron and the bioavailability of zinc. *British Medical Journal* **287**, 1013–1014.
Medeiros, D. M., Mazhan, A. & Brunett, E. W. (1987). Failure of oral zinc supplementation to alter hair zinc levels among healthy human males. *Nutrition Research* **7**, 1109–1115.
Menard, M. P. & Cousins, R. J. (1983). Zinc transport by brush border membrane vesicles from rat intestine. *Journal of Nutrition* **113**, 1434–1442.
Milne, D. B., Canfield, W. K., Mahalko, J. R. & Sandstead, H. H. (1983). Effect of dietary zinc on whole body surface loss of zinc: impact on estimation of zinc retention by balance method. *American Journal of Clinical Nutrition* **38**, 181–186.
Molokhia, M., Sturniolo, G., Shields, R. & Turnberg, L. A. (1980). A simple method for measuring zinc absorption in man using a short-lived isotope (^{69m}Zn). *American Journal of Clinical Nutrition* **33**, 881–886.
Mooradian, A. D. & Morley, J. E. (1987). Micronutrient status in diabetes mellitus. *American Journal of Clinical Nutrition* **45**, 877–895.
National Academy of Sciences (1980). *Recommended Dietary Allowances*, 9th ed. Washington, DC: National Research Council.
Navert, B., Sandstrom, B. & Cederblad, A. (1985). Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. *British Journal of Nutrition* **53**, 47–53.
Patrick, J. & Dervish, C. (1984). Leukocyte zinc in the assessment of zinc status. *CRC Critical Reviews in Clinical Laboratory Sciences* **20**, 95–114.
Pattison, S. E. & Cousins, R. J. (1986). Zinc uptake and metabolism by hepatocytes. *Federation Proceedings* **45**, 2805–2809.
Payton, K. B., Flanagan, P. R., Stinson, E. A., Chodirker, D. P., Chamberlain, M. J. & Valberg, L. S. (1982). Technique for determination of human zinc absorption from measurement of radioactivity in a fecal sample or the body. *Gastroenterology* **83**, 1264–1270.
Peirce, P. L., Hambidge, K. M., Goss, C. H., Miller, L. V. & Fennessey, P. V. (1987). Fast atom bombardment mass spectrometry for the determination of zinc stable isotopes in biological samples. *Analytical Chemistry* **59**, 2034–2037.
Pilch, S. M. & Senti, F. C. (1985). Analysis of zinc data from the second national health and nutrition examination survey (NHANES II). *Journal of Nutrition* **115**, 1393–1397.
Prasad, A. S. (1984). Discovery and importance of zinc in human nutrition. *Federation Proceedings* **43**, 2829–2834.
Prasad, A. S. (1985). Clinical manifestations of zinc deficiency. *Annual Review of Nutrition* **5**, 341–363.
Qvist, I., Abdulla, M., Jagerstad, M. & Svensson, S. (1986). Iron, zinc and folate status during pregnancy and two months after delivery. *Acta Obstetrica et Gynecologica Scandinavica* **65**, 15–22.
Record, I. R., Record, S. J., Dreosti, I. E. & Rohan, T. E. (1985). Dietary zinc intake of pre-menopausal women. *Human Nutrition: Applied Nutrition* **39A**, 363–369.
Ruz, M. (1984). Recommended zinc intake for the first six months of life. *Nutrition Research* **4**, 923–927.
Sandstead, H. H. (1985). Zinc: essentiality for brain development and function. *Nutrition Reviews* **43**, 129–137.
Sandstrom, B., Cederblad, A., Kivisto, B., Stenquist, B. & Andersson, H. (1986). Retention of zinc and calcium from the human colon. *American Journal of Clinical Nutrition* **44**, 501–504.
Sandstrom, B., Davidsson, L., Lundell, L. & Olbe, L. (1987*a*). Zinc status and dark adaptation in patients subjected to total gastrectomy: effect of zinc supplementation. *Human Nutrition: Clinical Nutrition* **41C**, 235–242.
Sandstrom, B., Kivisto, B. & Cederblad, A. (1987*b*). Absorption of zinc from soy protein meals in humans. *Journal of Nutrition* **117**, 321–327.

Scholmerich, J., Krauss, E., Wietholtz, H., Kottgen, E., Lohle, E. & Gerok, W. (1987). Bioavailability of zinc from zinc–histidine complexes. II. Studies on patients with liver cirrhosis and the influence of the time of application. *American Journal of Clinical Nutrition* **45**, 1487–1491.

Seal, C. J. & Heaton, F. W. (1987). Zinc transfer among proteins in rat duodenum mucosa. *Annals of Nutrition and Metabolism* **31**, 55–60.

Sheldon, W. L., Aspillaga, M. O., Smith, P. A. & Lind, T. (1985). The effects of oral iron supplementation on zinc and magnesium levels during pregnancy. *British Journal of Obstetrics and Gynaecology* **92**, 892–898.

Simmer, K., Iles, C. A., Slavin, B., Keeling, P. W. N. & Thompson, R. P. H. (1987*a*). Maternal nutrition and intrauterine growth retardation *Human Nutrition: Clinical Nutrition* **41**C, 193–197.

Simmer, K., Iles, C. A., James, C. & Thompson, R. P. H. (1987*b*). Are iron–folate supplements harmful? *American Journal of Clinical Nutrition* **45**, 122–125.

Simmer, K. & Thompson, R. P. H. (1985). Maternal zinc and intrauterine growth retardation. *Clinical Science* **68**, 395–399.

Simmer, K. & Thompson, R. P. H. (1986). Zinc and the fetus. *Journal of the Royal Society of Health* **5**, 166–168.

Solomons, N. W. (1979). On the assessment of zinc and copper nutriture in man. *American Journal of Clinical Nutrition* **32**, 856–871.

Solomons, N. W. (1982). Biological availability of zinc in humans. *American Journal of Clinical Nutrition* **35**, 1048–1075.

Solomons, N. W. & Cousins, R. J. (1984). Zinc. In *Absorption and Malabsorption of Mineral Nutrients*, pp. 125–197 [N. W. Solomons and I. H. Rosenberg, editors]. New York: Alan R. Liss.

Solomons, N. W. & Jacobs, R. A. (1981). Studies on the bioavailability of zinc in man. IV. Effect of heme and nonheme iron on the absorption of zinc. *American Journal of Clinical Nutrition* **34**, 475–481.

Solomons, N. W., Marchini, J. S., Duarte-Favaro, R., Vannuchi, H. & Dutra de Oliveira, J. E. (1983). Studies on the bioavailability of zinc in humans: intestinal interaction of tin and zinc. *American Journal of Clinical Nutrition* **37**, 566–571.

Song, M. K. (1987). Low-molecular-weight zinc-binding ligand: a regulatory modulator for intestinal zinc transport. *Comparative Biochemistry and Physiology* **87**A, 223–230.

Spencer, H., Kramer, L. & Osis, D. (1983). Zinc balances in humans during different intakes of calcium and phosphorus. In *Nutritional Bioavailability of Zinc. American Chemical Society Symposium Series* no. 210, pp. 223–232 [G. E. Inglett, editor]. Washington, DC: American Chemical Society.

Sullivan, J. F. & Lankford, H. G. (1965). Zinc metabolism and chronic alcoholism. *American Journal of Clinical Nutrition* **17**, 57–63.

Swanson, C. A. & King, J. C. (1987). Zinc and pregnancy outcome. *American Journal of Clinical Nutrition* **46**, 763–771.

Swanson, C. A., Turnland, J. R. & KIng, J. C. (1983). Effect of dietary zinc sources and pregnancy on zinc utilization in adult women fed controlled diets. *Journal of Nutrition* **113**, 2557–2567.

Takagi, Y., Okada, A., Itakura, T. & Kawashima, Y. (1986). Clinical studies of zinc metabolism during total parenteral nutrition as related to zinc deficiency. *Journal of Parenteral and Enteral Nutrition* **10**, 195–202.

Taper, L. J., Oliva, J. T. & Ritchey, S. J. (1985). Zinc and copper retention during pregnancy: the adequacy of prenatal diets with and without supplementation. *American Journal of Clinical Nutrition* **41**, 1184–1192.

Thomas, A. J., Bunker, V. W., Brennan, E. & Clayton, B. E. (1986). The trace element content of hospital meals and potential low intake by elderly patients. *Human Nutrition: Applied Nutrition* **40**A, 440–446.

Thomas, A. J., Bunker, V. W., Hinks, L. J., Sodha, N., Mullee, M. A. & Clayton, B. E. (1988). Energy, protein, zinc and copper status of twenty-one elderly inpatients: analysed dietary intake and biochemical indices. *British Journal of Nutrition* **59**, 181–191.

Thornalley, P. J. & Vasak, M. (1985). Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochimica et Biophysica Acta* **827**, 36–44.

Turnland, J. R., Durkin, N., Costa, F. & Margen, S. (1986). Stable isotope studies of zinc absorption and retention in young and elderly men. *Journal of Nutrition* **116**, 1239–1247.

Turnland, J. R., King, J. C., Keyes, W. R., Gong, B. & Michel, M. C. (1984). A stable isotope study of zinc absorption in young men: effects of phytate and α-cellulose. *American Journal of Clinical Nutrition* **40**, 1071–1077.

Tuttle, S., Aggett, P. J., Campbell, D. & MacGillivray, I. (1985). Zinc and copper nutrition in human pregnancy: a longitudinal study in normal primigravidae and in primigravidae at risk of delivering a growth-retarded baby. *American Journal of Clinical Nutrition* **41**, 1032–1041.

Vanderkooy, P. D. S. & Gibson, R. S. (1987). Food consumption patterns of Canadian preschool children in relation to zinc and growth status. *American Journal of Clinical Nutrition* **45**, 609–616.

Wada, L., Turnland, J. R. & King, J. C. (1985). Zinc utilization in young men fed adequate and low zinc intakes. *Journal of Nutrition* **115**, 1345–1354.

Wallwork, J. C. (1987). Zinc and the central nervous system. *Progress In Food and Nutrition Science* **11**, 203–247.

Walravens, P. A., Hambidge, K. M. & Koepfer, D. (1988). Zinc supplements in infants with failure to thrive: effects on growth. In *Trace Element Metabolism in Animals and Man* – 6 [L. S. Hurley, B. Lonnerdale, C. L. Keen and R. Rucker, editors]. New York: Plenum. (In the Press).

Ward, N. I., Watson, R. & Bryce-Smith, D. (1987). Placental element levels in relation to fetal development for obstetrically 'normal' births: a study of 37 elements. Evidence for effects of cadmium, lead and zinc on fetal growth, and for smoking as a source of cadmium. *International Journal of Biosocial Research* **9**, 63–81.

Wastney, M. E., Aamodt, R. L., Rumble, W. F. & Henkin, R. I. (1986). Kinetic analysis of zinc metabolism and its regulation in normal humans. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **20**, R398–R408.

Watson, W. S., McLauchlan, G., Lyon, D. T. B., Pattie, I. & Crean, G. P. (1988). Zinc absorption in pancreatic insufficiency. In *Trace Element Metabolism in Animals and Man* – 6 [L. S. Hurley, B. Lonnerdale, C. L. Keen and R. Rucker, editors]. New York: Plenum. (In the Press).

Weismann, K. & Hoyer, H. (1985). Serum alkaline phosphatase and serum zinc levels in the diagnosis and exclusion of zinc deficiency in man. *American Journal of Clinical Nutrition* **41**, 1214–1219.

World Health Organization (1973). *Trace Elements in Human Health*, p. 13. Geneva: WHO.

Zimmerman, A. W., Dunham, B. S., Nochimson, D. J., Kaplan, B. M., Clive, J. M. & Kunkel, S. L. (1984). Zinc transport in pregnancy. *American Journal of Obstetrics and Gynecology* **149**, 523–529.

Printed in Great Britain