EARLY BIOCHEMICAL DEFECTS CAUSED BY DIETARY TRACE ELEMENT DEFICIENCIES

J. K. CHESTERS AND J. R. ARTHUR

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

CONTENTS

INTRODUCTION

Many of the early studies of the metabolism of the trace elements were aimed at providing methods of assessing the adequacy of intake of individual elements. This may be achieved either by estimating body reserves of the elements or by determining whether their essential functions are being adequately maintained. Newly-evolving methods of assessing reserves include, for zinc, studies of metallothionein concentrations in blood or urine (Bremner *et al.* 1987) and, for iron, immunoassay of plasma ferritin levels (Peter & Wang, 1981). However, since trace element reserves are only of value in ensuring adequate functional capacity, methods based on the former approach must be calibrated against functional criteria. Furthermore, it has become increasingly evident that adverse biochemical changes can occur before diagnostic signs of trace element deficiencies are apparent. Such defects include reduced candidacidal activity of neutrophils from copper and selenium-deficient rats, mice and cattle (Boyne & Arthur, 1981, 1986*a*), degenerative changes in pancreas of cattle with low Cu intakes (Fell *et al.* 1985) and restricted growth of many species when Zn intakes are inadequate. Increasingly, therefore, investigations will concentrate on the functional roles of the trace elements with a view to being able to identify subclinical deficiencies. In the past, investigators frequently studied the multiple roles of individual elements, but to understand their significance each must be seen as an integral part of a particular metabolic pathway. For this reason, the present review will attempt to indicate how the trace elements function within an animal's overall metabolism.

The reference in the title to early defects relates to the time-course of onset of a deficiency and it should be recognized throughout that this may correspond to depletion for only a few days in the case of Zn but at the other extreme may extend with manganese deficiency into a second generation.

ENZYMES

The nutritional requirements for trace elements have frequently been attributed to their function as essential components of a range of enzymes. The only clearly-defined functional role for Se in animals is as part of the active centre of glutathione peroxidase (*EC* 1 . 11 . 1 . 9; GSHPx). Because the activity of this enzyme responds rapidly to alterations in dietary Se intake, it provides probably one of the best links between the intake of a trace element and its functional adequacy (Hafeman *et al.* 1974). Recently, evidence has been obtained that the carbon skeleton of the selenocysteine moiety in this protein is derived from serine and the seleno-amino acid is uniquely coded for by the triplet TGA (Chambers *et al.* 1986; Sunde & Evenson, 1987). This triplet normally acts as a stop codon and there will undoubtedly be much interest in what controls the interpretation of the codon during translation. Interestingly immunological evidence suggests that loss of GSHPx activity during the onset of Se deficiency is faster than loss of the protein (Knight & Sunde, 1987). However, once the deficiency has become established, the activity of the enzyme seems to be directly related to the amount of peroxidase-protein present (Takahashi *et al.* 1986). Possibly there is a transient state accompanying a reduction in Se status during which enzyme activity is lost more rapidly than the protein is degraded.

Although many of the effects of Se deficiency can be attributed to loss of Se-containing GSHPx activity, some of the consequences of the deficiency may be due to hitherto unidentified functions for Se (Burk, 1983; Reiter & Wendel, 1983, 1985; Hill *et al.* 1987; Arthur *et al.* 1988 *b*). This has led to interest in the identification of several seleno-proteins to which no specific functions have as yet been assigned (Sunde, 1984; Combs & Combs, 1986; Evenson & Sunde, 1988). Probably the most-intensively studied of these non-GSHPx seleno-proteins is the 'P-protein' (molecular weight 75000–80000) found in plasma, liver and kidney (Burk & Gregory, 1982; Motsenbocker & Tappel, 1984). Recently, monoclonal antibodies to P-protein have been prepared and a radioimmunoassay used to quantify the effects of Se deficiency on levels of the protein in plasma (Yang *et al.* 1987). This protein may well have an important function since, on repletion of Se-deficient animals with Se, its concentration returns to normal before that of GSHPx.

Another newly-recognized consequence of Se deficiency is an elevation of the concentration of thyroxine (T4) and reduction in triiodothyronine (T3) in plasma in rats and cattle (Beckett *et al.* 1987; Arthur *et al.* 1988 *a*). This appears to stem from a reduced rate of conversion of T4 to the more-metabolically-active T3 due to lowered activity of the hepatic microsomal deiodinase (Beckett *et al.* 1987). Interestingly both a hypothyroid state and severe Se deficiency result in increased activities of a number of glutathione S-transferases (*EC* 2 . 5 . 1 . 18; GST) (Arias *et al.* 1976; Arthur *et al.* 1987). Since certain of the latter have peroxidase activities with organic hydroperoxides similar to that of GSHPx, it has been suggested that their activity is enhanced in Se deficiency to compensate for loss of GSHPx. However, investigation of the response of individual GST has now shown that their activities increase to comparable extents independently of their ability to act as peroxidases (Arthur *et al.* 1987). Furthermore, the increases in GST activity do not appear to be secondary to an induced hypothyroidism since these increases could be reversed by small doses of Se which did not ameliorate the defective T4 metabolism (Reiter & Wendel, 1984; Arthur *et al.* 1988 *b*).

Zn is recognized as a component of numerous enzymes, examples being found in each of the main classes (Vallee & Galdes, 1984). However, although it seemed likely that the effects of Zn deficiency would be attributable to loss of a Zn metalloenzyme and some, for example, plasma alkaline phosphatase (*EC* 3 . 1 . 3 . 1), and lactic dehydrogenase (*EC* 1 . 1 . 1 . 27) are reduced in activity sufficiently rapidly to be helpful in diagnosing the
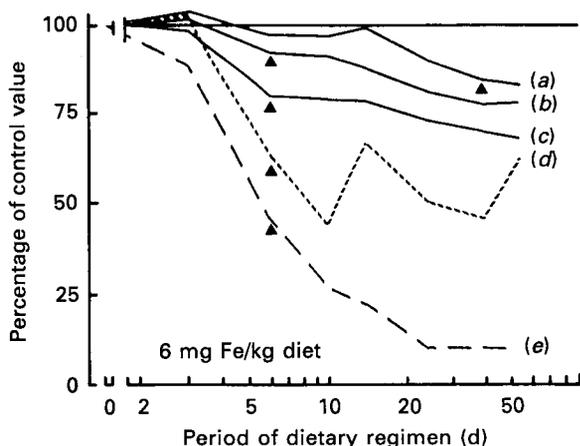
Fig. 1. Sequence of development of iron deficiency. (a) Muscle cytochrome c; (b) packed cell volume; (c) cytochrome c; (d) transferrin saturation; (e) liver Fe. (▲) The day on which differences between Fe-deficient and control groups first became significant ($P < 0.05$); thereafter the differences remained significant at all samplings.

deficiency in both animals and man (Kirchgessner & Roth, 1975; Prasad, 1982), none have yet been implicated in the failure of growth caused by lack of Zn. Illustrative of the situation is a reduction in angiotensin-converting enzyme (EC 3.4.15.1), a Zn dipeptidase, in Zn-deficient rats which nevertheless had normal concentrations of angiotensin II in their plasma (Reeves & O'Dell, 1986). Similarly, the activity of fructose-1, 6-bisphosphatase (EC 3.1.3.11) which contains twelve Zn per molecule and is a regulatory enzyme in gluconeogenesis, decreased by nearly 50% in the liver of rats within 1 d on a Zn-deficient diet. It could also be restored to normal within 30 min of Zn supplementation but the plasma glucose levels of the Zn-deficient animals were not affected (Cowen et al. 1986). In contrast, lowered plasma glucose values were observed in pups from dams reared on a Mn-deficient diet and these were associated with decreased activities of phosphoenolpyruvate carboxykinase (EC 4.1.1.49), a Mn-enzyme also involved in gluconeogenesis (Baly et al. 1984). Thus the effects of trace element deficiencies on an animal's metabolic pathways depend not only on the presence of metalloenzymes but also on the extent to which these enzymes control the flux of metabolites through the pathways.

Loss of Fe as a component of haem in haemoglobin, myoglobin and cytochromes probably accounts for most of the biological effects of Fe deficiency (Beard et al. 1984; Dallman, 1986). Fig. 1 illustrates a time-course of depletion of haem-Fe in rats offered an Fe-deficient diet and shows that losses occurred before Fe reserves were exhausted (Dallman et al. 1982).

Cu is a component of several enzymes which may lose activity in Cu deficiency and lead to biochemical and metabolic changes. At least five such enzyme systems are recognized as having possible relevance to the occurrence of defects in Cu deficiency (see Prohaska & Heller, 1982). Thus loss of cytochrome oxidase (EC 1.9.3.1) activity may affect mitochondrial metabolism, reduced superoxide dismutase (EC 1.15.1.1) will affect cell antioxidant systems, catecholamine metabolism can be altered by decreased dopamine-β-hydroxylase (EC 1.14.17.1), decreased lysyl oxidase activity may adversely influence collagen and elastin formation and loss of ferroxidase activity of caeruloplasmin (EC 1.16.3.1) may change Fe metabolism. Prohaska & Heller (1982) concluded that the

hypertrophy and abnormal functioning of hearts from Cu-deficient rats could arise from changes in at least three of these enzyme systems, resulting in alterations in the elastic properties of muscle, cardiac catecholamine depletion and defective energy metabolism, but the relative importance of each was not clear. Some enzyme changes in Cu deficiency and also cardiac hypertrophy are more severe in rats when a large proportion of dietary carbohydrate consists of fructose rather than glucose or starch (Reiser *et al.* 1983; Fields *et al.* 1984*a*). Thus changes in the overall composition of a diet not directly related to its mineral content may influence the outcome of a trace element deficiency.

ENERGY SUPPLY AND CELL METABOLISM

Dietary trace element deficiency can alter the intermediary metabolism of cells and their energy production. For instance Cu deficiency is known to decrease both liver and heart ATP concentrations (Kopp *et al.* 1983; Davies & Lawrence, 1986). In descriptions of the morphological effects of Cu deficiency attempts have been made to relate the changes, in particular the characteristic mitochondrial enlargement, to biochemical defects (Fell, 1981, 1987). Mitochondrial lesions, possibly caused by loss of cytochrome oxidase activity, are common to several of the histological defects seen in different organs in Cu deficiency (Fell, 1981). However, despite the morphological changes, in early and severe Cu deficiency the mechanical properties of hepatic mitochondria were not changed (Lawrence *et al.* 1985). Cu deficiency caused a small increase in the percentage of docosapentaenoic acid (22:5) in mitochondrial fatty acids without altering the unsaturation index (Lawrence *et al.* 1985). In vitro, hepatic mitochondrial state-three respiration (in the presence of ADP) decreased in Cu deficiency (Davies *et al.* 1985). However, this could not be explained by loss of cytochrome oxidase activity which was still adequate to sustain a greater rate of oxygen consumption in the presence of an uncoupler. Mitochondrial respiration seems to be decreased by impaired adenine nucleotide translocase activity secondary to a fall in total adenine nucleotide concentration (Davies & Lawrence, 1986).

Dietary Fe deficiency in rats resulted in reduced cytochrome concentrations in the intestine (Dallman *et al.* 1982) and lowered succinic dehydrogenase (*EC* 1.3.99.1) activity in skeletal muscle (Evans & Mackler, 1985; Quisumbing *et al.* 1985) before these enzymes were affected in liver, before Fe stores were exhausted and while the degree of anaemia was still relatively mild. This is associated with a lower $V_{O_2 max}$ (maximum rate of oxygen consumption) and impaired endurance of the muscles (Davies *et al.* 1984; Evans & Mackler, 1985). Cross-transfusion of blood between deficient and control rats to equalize haemoglobin concentrations reversed the defect in maximum O_2 consumption, but the restricted endurance of muscle was not improved (Davies *et al.* 1984).

Before Se deficiency causes any obvious clinical changes in an animal, several biochemical defects can be detected. In addition to those which can be related to loss of GSHPx, there are others whose origins are less clear. Pascoe *et al.* (1983) showed that within 1 d Se deficiency caused decreased intestinal, cytochrome P450-dependent, mixed-function oxidase (*EC* 1.14.14.1) activity. Other effects of Se on cell metabolism require a more-prolonged deficiency. Conversion of methionine to cysteine is impaired by Se deficiency in some strains of chickens (Bunk & Combs, 1981; Haplin & Baker, 1984). In mice despite no change in total exhalation of carbon dioxide there is increased $^{14}CO_2$ exhalation from [1–^{14}C]- or [6–^{14}C]glucose (Wendel & Otter, 1987). Otter *et al.* (1986) reported that after partial hepatectomy in mice there is a threefold increase in the rate of DNA synthesis in Se-deficient in comparison to Se-supplemented animals. These results were interpreted by Otter *et al.* (1986) as indicating that Se is involved at as yet unidentified sites in the control of intermediary metabolism. In addition Se deficiency can cause elevated hepatic haem

oxygenase (EC 1.14.99.3) activity (see Burk, 1983; Combs & Combs, 1986). Se deficiency also increases hepatic glutathione synthesis and release into the plasma and elevates plasma but not hepatic glutathione concentrations (Hill & Burk, 1985; Hill et al. 1987). Reversal of these effects on haem and glutathione metabolism during Se repletion occurs before changes in GSHPx are measurable (Burk, 1983; Reiter & Wendel, 1984; Hill et al. 1987). The combined evidence already described provides further support for a function(s) for Se in the control of intermediary metabolism separate from its role in GSHPx.

OXIDATIVE STRESS

Many reactions required for maintenance of normal metabolism and the production of energy in the cell produce potentially-toxic free radicals as unwanted by-products. This is especially a problem with pathways which utilize O_2. Trace elements play an important role in the many systems in the cell which have evolved to deal with free radicals (Fig. 2). As discussed previously, Se is an essential component of glutathione peroxidase which destroys hydrogen peroxide and many organic hydroperoxides that can lead to the generation of free radicals. However this enzyme will not metabolize phospholipid hydroperoxides which occur in cell membranes (Grossman & Wendel, 1983). A seleno-protein (Mr 23000) which can metabolize phospholipid hydroperoxides has been isolated from rat heart (Ursini et al. 1985). This protein may provide a hitherto unrecognized function of Se in the cell, although it could yet prove to be a subunit of GSHPx.

The vitamin E status of an animal can influence the consequences of Se deficiency. Heart homogenates from Se-deficient rats when incubated with Fe^{2+} produced the same amount of free radicals as Se-sufficient tissue. Only in the presence of a concurrent vitamin E deficiency did Se deficiency cause increased radical formation in incubated heart homogenates (Arthur et al. 1988c). Free-radical-mediated breakdown of polyunsaturated fatty acids can result in vivo in exhalation of the hydrocarbons ethane and pentane. Injection of Se- and vitamin E-deficient rats with Fe^{2+} caused an increase in ethane exhalation when compared with Se- and vitamin E-supplemented control animals (Dougherty et al. 1981). In Se- and vitamin E-deficient cattle increased intake of polyunsaturated fatty acid can cause myopathy (Kennedy et al. 1987), but this will not always develop in cattle of low Se and vitamin E status (Siddons & Mills, 1981, Arthur, 1988). Myopathies in patients undergoing total parenteral nutrition and the cardiomyopathy, Keshan Disease, endemic in humans in large areas of China are associated with low Se status and can be ameliorated by Se supplementation (Robinson & Thomson, 1983). However, the relationship of these conditions to vitamin E status is unclear.

Deficiencies of Cu and Mn can decrease respectively the activity of Cu–Zn and Mn-containing superoxide dismutases in liver and other tissues (Paynter et al. 1979; Paynter 1980a, b; Paynter & Martin 1980; Zidenberg-Cherr et al. 1983). These adverse effects on antioxidant enzymes may explain why Cu, Mn and Zn deficiencies have all been shown to increase the formation of free radicals or the products of free-radical-mediated damage to polyunsaturated fatty acids in various tissue homogenates and microsomal fractions (Paynter 1980b; Sullivan et al. 1980; Zidenberg-Cherr et al. 1983; Fields et al. 1984b; Bray et al. 1986; Hammermueller et al. 1987). Additionally, decreased tissue ascorbic acid concentrations in Cu deficiency may increase susceptibility to oxidative damage (Prohaska & Cox, 1983). Alternatively, the increase in free radicals and in the breakdown products of polyunsaturated fatty acids could possibly be a secondary consequence of tissue damage caused by the trace element deficiency (Arthur et al. 1988c). Therefore, it is important to demonstrate that these increases occur early in the deficiency as an indication that they are the cause rather than the consequence of pathological changes associated with the disease.
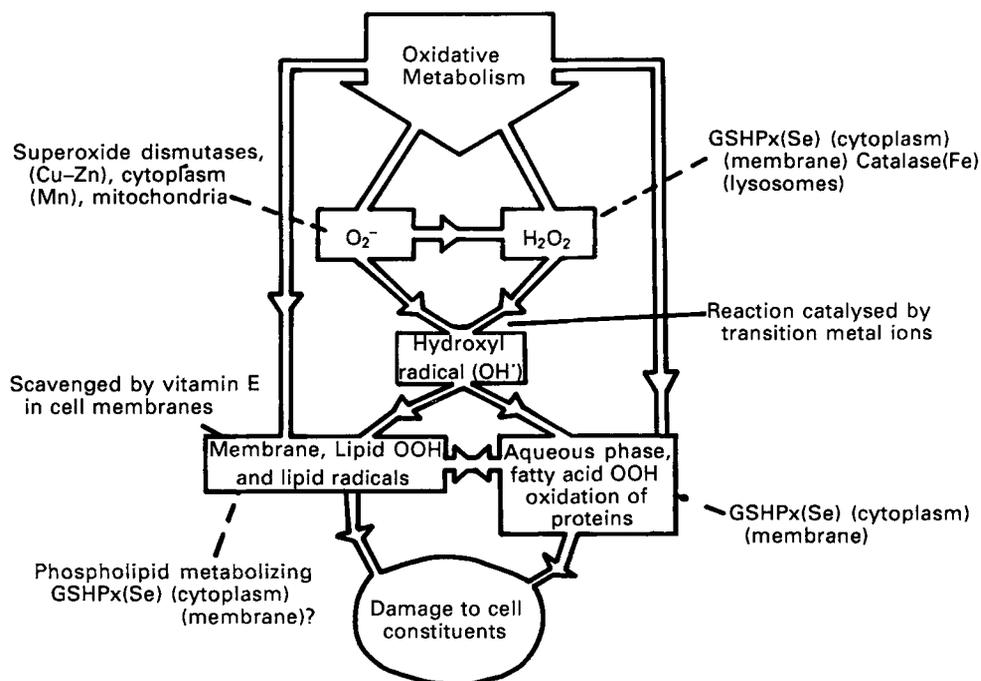
Fig. 2. Trace elements in cell antioxidant systems. Oxidative metabolism in the cell produces free radicals directly or derived from superoxide (O_2^-) and hydrogen peroxide via a transition metal catalysed Haber-Weiss reaction. (– – –), Trace-element-dependant enzymes are involved in the prevention of cell damage. GSHPx, glutathione peroxidase ($EC\ 1\ .\ 11\ .\ 1\ .\ 9$). Lipid OOH, lipid hydroperoxides; fatty acid OOH, fatty acid hydroperoxides. The trace element components of the different antioxidant enzymes are given in parentheses.

The demonstration *in situ* of increased chemiluminescence, probably due to elevated formation of oxygen derived free radicals, in liver from Se- and vitamin E-deficient rats before the occurrence of heptic necrosis has now provided strong evidence for a primary role for free radicals in the initiation of the disease (Fraga *et al.* 1987). However, chemiluminescence does not allow direct identification of the free radicals involved which may only be possible by using electron-spin-resonance spectroscopy.

Although trace element deficiencies generally affect cell antioxidant systems through an enzyme containing the element concerned, certain deficiencies will modify the activity of antioxidant enzymes not containing that element. Thus Cu deficiency can decrease the activity of the seleno-enzyme GSHPx in rat liver (Jenkinson *et al.* 1982; Arthur *et al.* 1987; Allen *et al.* 1988). Combined Se and vitamin E deficiencies will decrease Mn superoxide dismutase activity in rat pancreas as well as causing the expected changes in vitamin E concentrations and GSHPx activity (Asayama *et al.* 1986). Complex interactions between Cu, Mn, Se and vitamin E deficiencies have been described for the rat. These deficiencies can interact to increase the susceptibility of some tissues to peroxidation (Paynter 1980*b*).

LIPIDS AND EICOSANOIDS

Numerous investigators have studied the effects of Zn deficiency on polyunsaturated fatty acid metabolism and eicosanoid synthesis but the only consensus which appears to have emerged is that the results are very susceptible to changes caused by alterations in food

intake (Kramer *et al.* 1984; Fogerty *et al.* 1985; Bales *et al.* 1986). In Zn-deficient rats *ad lib.* food consumption is not only reduced, but daily intakes cycle from near normal to zero and back again, presenting severe difficulties in providing suitable control animals (Williams & Mills, 1970). Various strategies have been employed to control for these effects, but none can overcome the basic paradox that equalizing the food intake of a control rat with that of the deficient renders the former unable to grow because of inadequate intake of either protein or energy, whereas the Zn-deficient rat has adequate supplies of both. Clearly, this is liable to produce differences in the energy and lipid metabolism of the two groups independent of any direct effects of Zn. This is reflected in a series of investigations where there were no differences between *ad lib.*-fed groups regardless of their Zn intake, but the lipid metabolism of the groups fed *ad lib.* differed significantly from restricted or pair-fed controls (Hwang *et al.* 1984; Kramer *et al.* 1984; Bales *et al.* 1986). This suggests that Zn deficiency *per se* has little effect on lipid metabolism but that the associated reduction in food intake will modify that of pair-fed controls. At present it does not seem possible to draw clear conclusions regarding the effects of Zn deficiency on tissue lipid composition and metabolism. For example, Fogerty *et al.* (1985) reviewed seven investigations of the effects of Zn deficiency on Δ6-desaturase activity. In three of these its activity was increased in the low-Zn animals and in the other four, reduced. Values for arachidonic acid content of liver lipids were given in four of these reports, two indicating no effect of Zn deficiency, one an increased concentration of this fatty acid and the fourth a decrease. Fogerty *et al.* (1985) suggested, however, that some of these discrepancies may reflect different effects on tissue and membrane lipids.

In contrast to the previous discussion, there does appear to be agreement that the synthesis of prostaglandins and thromboxanes is not impaired by Zn deficiency (Gordon *et al.* 1983; Hwang *et al.* 1984). These authors disagreed, however, on the extent to which the deficiency alters platelet aggregation. There is evidence to suggest that the numbers and affinities of prostaglandin receptors are modified by lack of Zn (Gordon *et al.* 1983; Li & O'Dell, 1986) and it is possible that defects in platelet aggregation may result from reduced receptor sensitivity to the eiconsanoids (Li & O'Dell, 1986).

Se has a direct role in the lipid metabolism of platelets where GSHPx converts 12-hydroperoxy 5,8,11,14 eicosatetraenoic acid (12-HPETE) to the corresponding hydroxy acid, 12-HETE (Bryant *et al.* 1983). Products of 12-HPETE degradation as well as lipid hydroperoxides, all formed when GSHPx activity is low, can inhibit prostacyclin synthesis (Masukawa *et al.* 1983; Funk *et al.* 1987). Thus decreased prostacyclin synthesis in platelets and aorta of rats is likely to contribute to increased platelet aggregation observed in Se deficiency (Masukawa *et al.* 1983).

Hypercholesterolaemia is the most-widely-studied effect of Cu deficiency on lipid metabolism and this has been postulated to contribute to the incidence of coronary heart disease (for review, see Samman & Roberts 1985). However marginal Cu deficiency in utero and then for a further 117 d caused ultrastructural damage to aorta in male rats without altering plasma or aortal cholesterol concentrations (Hunsaker *et al.* 1984). Possible effects of Cu deficiency contributing to coronary heart disease are thus not confined to changes in cholesterol metabolism.

GROWTH AND CELL REPLICATION

The most-consistent early effect of Zn deficiency in rats is a reduction in growth which is generally associated with a fall in voluntary food intake sufficient to produce a comparable growth check in Zn-adequate rats given similar amounts of food (Chesters & Quarterman, 1970). However, previous investigations had indicated and recent results confirm that

force-feeding normal quantities of the deficient diet, far from reinstating growth, actually results in a rapid decline in the health of the rats (Chesters & Quarterman, 1970; Park *et al.* 1985). Furthermore, McClain *et al.* (1985) have shown that administration of agents which normally stimulate food intake failed to overcome the effects of Zn deficiency. Also alterations in brain noradrenaline concentrations (Kasarskis *et al.* 1986) and plasma amino acid patterns (Wallwork *et al.* 1981) seen in Zn-deficient rats appeared to result from changes in food intake rather than initiating these changes. It thus appears that failure of growth in Zn-deficient rats results from a biochemical defect rather than from undernutrition caused by reduced appetite.

As mentioned previously, attempts to link growth failure to loss of a specific Zn-enzyme have so far proved unsuccessful. Previous investigations indicated that protein synthesis was less sensitive to lack of Zn than synthesis of new cells, as indicated by thymidine incorporation (Williams & Chesters, 1970). Although it has recently been reported that protein synthesis was impaired in a cell-free system isolated from the liver of Zn-deficient rats (Hicks & Wallwork, 1987), carefully-controlled studies in vivo indicated that in muscle most and possibly all the reduction in protein synthesis in Zn-deficient rats could be ascribed to decreased food intake (Giugliano & Millward, 1987). In a separate study, there was a lower mitotic index in jejunal cells of Zn-deficient rats but the rate of jejunal protein synthesis was not affected (Southon *et al.* 1985). Examination of the intestines of animals force-fed a Zn-deficient diet indicated that they were of normal weight but reduced DNA content (Park *et al.* 1985). Current evidence therefore points to a critical role of Zn in cell replication rather than protein synthesis.

The possibility that the reduced growth of Zn-deficient animals results from lower levels of growth hormone had been investigated, but supplementation with growth hormone did not reverse the defect (Prasad *et al.* 1969). Although somatomedin concentrations were lowered by Zn deficiency (Cossack, 1986), they were not returned to normal by administration of growth hormone (Oner *et al.* 1984), nor were they significantly lower than in Zn-adequate rats whose food intake was restricted to match accurately that of the deficient animals (Bolze *et al.* 1987).

Normal differentiation of fetal tissues was extremely sensitive to even transient reductions in Zn supply (Dreosti *et al.* 1985) and the lining of the forestomach failed to keratinize correctly in Zn-deficient rats (Ng *et al.* 1984). In man, Zn deficiency resulted in histological changes in the epidermis consistent with slower differentiation of the cells migrating from the basal layer (Ortega *et al.* 1985), a pattern also seen in the parakeratotic tissues of the buccal mucosa and oesophagus of Zn-deficient rats and rabbits (Joseph *et al.* 1981; Gerson *et al.* 1983). Significantly, the mitotic index of the latter tissues was elevated by the deficiency even though their Zn content was reduced to an even greater extent than that of the body as a whole (Gerson *et al.* 1983). Chen (1986) has now shown that the high mitotic index in Zn-deficient buccal mucosa results not from delayed passage of the cells through mitosis but from a shortened G_1 phase, all other phases of the cell cycle being of normal duration. These observations support the previous suggestion that the effects of Zn deficiency on growth can best be explained by a need for Zn to facilitate the changes in genetic expression required for induction of DNA synthesis and differentiation in normal cells (Chesters, 1978). A similar conclusion has been drawn regarding the function of Zn in the protist, *Euglena gracilis* (Crossley *et al.* 1982).

In this context, the discovery of a class of gene-regulatory proteins containing a common structure, the 'Zn finger', is of great potential significance to our understanding of the effects of Zn deficiency (for review, see Klug & Rhodes, 1987). As first characterized in the protein TFIIIA, a regulator of 5S rRNA transcription, these consist of loops of amino acids which are structured by Zn acting as a bridge between two pairs of cysteine or histidine

residues located one on each side of the base of the loop. TFIIIA contains possibly as many as eleven of these 'fingers' which are thought to project into the major groove of the DNA and provide the specificity for the protein's binding to the promoter sequence in the gene. Only in TFIIIA and in the bacterial protein g32P have sufficient quantities of the proteins become available to demonstrate the presence of Zn unequivocally, but many other regulatory proteins including several steroid receptors have been shown to have amino acid sequences consistent with the formation of 'Zn fingers'. With TFIIIA, Zn has been shown to be essential for its function in purified preparations, cell-free extracts (Hanas *et al.* 1983; Wingender *et al.* 1984; Miller *et al.* 1985) and recently phytohaemagglutinin-stimulated lymphocytes, but investigations with Zn-deficient rats indicated that the restriction of Zn availability induced by dietary deficiency was insufficient to inhibit 5S rRNA synthesis (Chesters, unpublished results). This does not, however, preclude a role for other members of this group of regulatory proteins in the restriction of growth of Zn-deficient rats.

Another possible function for Zn in mediating cell division could be in the synthesis of diadenosine tetraphosphate (Bambara *et al.* 1985; Baril *et al.* 1985). This dinucloetide has been postulated to act as a second messenger of mitotic induction (Grummt *et al.* 1986) and its only known route of synthesis is via a Zn-mediated modification of the action of certain amino acyl transferases (Blanquet *et al.* 1983).

Although Fe deficiency has been shown to reduce the mitotic index of the jejunal mucosa (Perkkio *et al.* 1987) and lack of Mn can inhibit both growth and glycosaminoglycan synthesis (Bolze *et al.* 1985), neither deficiency has yet been clearly implicated in the control of cell replication.

REPRODUCTION

Deficiencies of either Zn or Mn can cause fetal abnormalities in rats but the nature of these and their relationship to the period of deficiency differ. Poor survival and ataxia in the pups are characteristics of low Mn intake but only when the dams have been raised from weaning on a Mn-deficient diet (for review, see Hurley, 1981). The ataxia results from low activities of Mn-dependent transglycosylases leading to impaired mucopolysaccharide synthesis in the developing otoliths and can be prevented by injecting the dams with Mn on day 14 of gestation. Delaying Mn treatment until day 16 improved survival but did not prevent ataxia. In contrast, a Zn-deficient diet will still induce fetal abnormalities when offered only from d 0 of gestation onwards. Maximum sensitivity to lack of Zn occurs between d 6 and d 12, and Record *et al.* (1986) have shown that within this period, the small variations in Zn availability induced by high or low intake of a Zn-deficient diet on a particular day are sufficient to determine whether the tissues differentiating on that day develop normally. Restricting the intake of a low-Zn diet reduces its teratogenic effects by inducing catabolic release of Zn mainly from soft tissues, but in early gestation some Zn may be derived from bone since low-calcium diets are effective in limiting teratogenicity (Masters *et al.* 1983, 1986).

Lack of Zn at the end of gestation results in delayed and prolonged parturition in rats (Hurley, 1981). This appears to be caused by the contractions of the uterine smooth muscles being weak, irregular and poorly synchronized (Lytton & Bunce, 1986). Underlying these defects is a failure to achieve the transfer from progesterone to oestrogen dominance, which normally occurs at the end of parturition, even though the alterations in circulating levels of the two hormones were still unaffected by the deficiency as late as d 22 of gestation. Since the oestrogen receptor has been shown to contain putative 'Zn fingers' of the type discussed previously (Klug & Rhodes, 1987), it is tempting to suggest that the disturbances of parturition in Zn deficiency result from a failure to synthesize adequate quantities of these proteins to allow normal induction of oestrogen-mediated changes.

Lack of Zn can also cause reproductive problems in males. In rats, failure of spermatids to transform into spermatozoa, a process requiring major changes in chromatin condensation and genetic expression, was shown to be impaired after 23 d on a Zn-deficient diet which resulted in an inhibition of growth within the first week (Orgebin-Crist *et al.* 1971). Zn deficiency in rams has been reported to cause a failure of spermatogenesis (Underwood & Somers, 1969) and oligospermy is also characteristic of Zn deficiency in man where it is associated with reduced testosterone levels (Prasad, 1982).

IMMUNITY

Plasma Fe and Zn concentrations are reduced by infection or acute stress, both effects being mediated by release of interleukin-I and following similar time-courses. However, whereas the loss of Zn from plasma was independent of blood granulocyte numbers, the reduction in plasma Fe level depended on granulocytes, possibly through release of lactoferrin by these cells (Goldblum *et al.* 1987).

The fall in plasma Fe and Zn concentrations during infection may be beneficial to the animal (Chesters & Will, 1981; Weinberg, 1984) but deficiency of either element adversely affects an animal's immune response (Fraker *et al.* 1986; Dallman, 1987). Lack of Fe reduces interleukin-I production (Helyar & Sherman, 1987) and decreases the 'oxidative burst' of neutrophils, probably via lower concentrations of the Fe-enzyme myeloperoxidase (Dallman, 1986; Murakawa *et al.* 1987). Reduced activity of another Fe-enzyme, ribonucleotide reductase (EC 1.17.4.1), which is required for synthesis of DNA precursors, may be responsible for depressed cell-mediated immunity observed in Fe-deficient humans, but not during stimulation in vitro of lymphocytes from these patients (Dallman, 1987). Unlike most Fe enzymes, ribonucleotide reductase has a relatively-low affinity for Fe and its activity will be lost readily as Fe availability declines in vivo, but in cultures it may be reactivated by adventitious Fe present in the medium. Humoral immunity appears relatively little affected by either Fe or Zn deficiency, but the latter does inhibit the cell-mediated response possibly through loss of the Zn-dependent peptide, thymulin, which is required for the differentiation of T-lymphocytes (Dardenne *et al.* 1982, 1984; Fraker *et al.* 1984; Mercalli *et al.* 1984). This view is strengthened by the observation that when T-lymphocyte numbers were reduced by Zn deficiency the proportion of T-helper cells within the population remained unaltered, suggesting a uniform effect on the differentiation of all types of T-cell (Dowd *et al.* 1986). Alternative suggestions that alterations in adrenalcorticoid secretion are responsible for the impaired T-cell function seen in Zn deficiency seem to have been at least partially discounted by studies in which loss of T-cell function occurred in advance of increase in plasma glucocorticoids and even after adrenalectomy (De Pasquale-Jardieu & Fraker, 1980).

Investigations of the effects of dietary Fe and Zn deficiencies on response to infection have produced confusing results which may be at least partially explicable by conflicting effects of the deficiencies (Kuvibidila, 1987). Thus reduced concentrations of these elements in plasma may impair bacterial growth (Weinberg, 1984) or reduce the severity of the stress response to endotoxin (Chesters & Will, 1981), but if they also result in a reduced immune response to invading organisms, the balance of benefit and loss to the animal would be hard to predict and could explain the variation in results observed in different experiments and with different organisms.

Both Se and Cu deficiencies can impair the candidacidal activity in vitro of neutrophils and macrophages from a variety of animal species (Serfass & Ganther, 1975; Boyne & Arthur, 1979, 1981, 1986a, b; Jones & Suttle, 1981; Boyne *et al.* 1986). These changes have been attributed to the loss of antioxidant enzymes allowing toxic oxygen derived species

produced in the phagocytic cells to cause the cells own demise (Boyne & Arthur, 1979; Arthur *et al.* 1981; Jones & Suttle, 1981; Baker & Cohen, 1984). However impaired neutrophil function can be detected at a very-early stage of Cu deficiency, before falls in Cu–Zn superoxide dismutase activity can be detected, suggesting in this case mechanisms other than loss of antioxidant activity must be involved (Arthur & Boyne, 1985). An important factor to consider in studying the effects of trace element deficiency on neutrophil function is that restriction of food intake can decrease neutrophil microbicidal activity (Boyne & Arthur, 1986*b*). Thus trace elements can not always be claimed to have a specific function in neutrophils when impaired function is accompanied by alterations in food intake and growth.

In Se deficiency the impaired neutrophil microbiocidal activity described previously is not always observed. Thus in Se-deficient rat neutrophils which had impaired candidacidal activity compared with Se-supplemented controls, killing of ingested *Salmonella typhimurium* and *Staphylococcus aureus* was not affected (Boyne *et al.* 1986). The reason for this may be that killing of the bacteria placed less oxidative stress on the antioxidant-deficient neutrophil than the greater metabolic activity involved in the killing of the much-larger yeast cells. This increased activity could have included the production of sufficient oxygen derived radicals to impair the function of the neutrophil as well as the yeast.

Apart from affecting neutrophil function Cu and Se deficiencies can impair other functions of the immune system. Even with a very-mild Cu deficiency there were decreased numbers of antibody-producing cells in the spleen which correlated with lowered plasma caeruloplasmin activity (Prohaska & Lukasewycz, 1981). More recently Cu deficiency has been demonstrated to alter the composition of splenocyte-membrane fatty acids which may possibly explain how Cu deficiency can cause some of the alterations in immune response (Korte & Prohaska, 1987). Other effects of both Se and Cu deficiencies on the immune system are to impair the response of lymphocytes to B- and T-cell mitogens (Lukasewycz & Prohaska, 1983; Parnham *et al.* 1983; Eskew *et al.* 1985) and reduce antibody production and natural killer-cell activity (Koller *et al.* 1987).

With the many adverse effects on the immune system it is hardly surprising that a subclinical Cu deficiency will increase the susceptibility of mice to a *Pasteurella haemolytica* infection (Jones & Suttle, 1983). However, the responses of Se-deficient rats and mice to other infections are much less clear cut. Se deficiency increased the susceptibility of mice to *Candida albicans* infection and of rats to *S. aureus* infection (Boyne & Arthur, 1986*a*; Boyne *et al.* 1986), whereas the susceptibility of rats to *S. typhimurium* and mice to *Listeria monocytogenes*, *Plasmodium bergii* or *Pseudorabies* virus were decreased (Boyne *et al.* 1984; Murray & Murray, 1985). One explanation for these complex responses to Se deficiency may be similar to that for either increased or decreased response to infection in Fe deficiency. The growth of some organisms may be more-severely inhibited by Se deficiency than the immune system of the host, either directly or by a change in the host-cell metabolism. Alternatively the increased susceptibility of mice to *C. albicans* infection may reflect the importance of the neutrophil in dealing with this infection (Boyne & Arthur, 1986*a*).

FUTURE PROSPECTS

Although much is now known of the functions of trace elements in the metabolism of animals, there are still many aspects awaiting clarification. Some of these have been highlighted here and one which may be of wide ranging significance is the role of Zn in cell replication and differentiation. Important advances in this field seem likely to stem from studies of cell-free systems or cells in culture, but assessment of the relationship of such observations to defects occurring in Zn-deficient animals must take into account the

relative levels of free Zn in the different experimental situations. Earlier computer simulations (May *et al.* 1977) and recently direct estimation (Magneson *et al.* 1987) indicate that in normal animals the free Zn^{2+} concentration in plasma is about 2×10^{-10} M. Both approaches suggest that this will vary directly with the total exchangeable Zn, and Zn-deficient animals may therefore have between one-third and one-tenth this concentration of free Zn^{2+}. In the past many investigations of the effects of Zn in vitro or in cultures have used added concentrations as high as 10^{-3} M. While the free Zn^{2+} concentration will in most cases be only a small fraction of the total added, nevertheless in many instances it must have been far higher than could be expected to occur in vivo. Equally recent investigations have shown that while loss of TFIIIA function because of lack of Zn inhibited 5S rRNA synthesis in EDTA-treated lymphocytes, this did not occur in Zn-deficient rats where the degree of deficiency was probably less severe (Chesters, unpublished results). In future, care must be taken with experimental design to ensure that the availability of Zn in vitro matches that in vivo if the results obtained are to be used to explain events happening in the whole animal.

Much has still to be discovered regarding the interactions of Se and vitamin E on the one hand and Cu and Fe on the other in maintaining adequate aerobic respiration without allowing excessive tissue damage by oxygen derived free radicals. For example, studies have recently revealed that plasma glutathione peroxidase is immunologically distinct from that found in liver and the cellular components of whole blood (Takahashi & Cohen, 1986). The origin of the plasma protein and its functional significance remain to be clarified. Also in future studies of antioxidant defects associated with trace element deficiencies, care must be taken to establish that increased production of free radicals is a primary effect of the deficiency rather than a secondary consequence of tissue damage.

In addition to the elements which have already received much attention, others are now emerging as important components of an animal's metabolic pathways. Recent studies of boron have indicated that differences in intake which are probably within the normal range may influence plasma oestradiol and testosterone concentrations in post-menopausal women (Nielsen *et al.* 1987). Furthermore nickel is known to be a component of several enzymes (Walsh & Orme-Johnson, 1987). Although none of these are of mammalian origin the element may well be important at least in ruminants where it is present in urease (*EC* 3.5.1.5) of rumen micro-organisms (Spears, 1984).

There is still a long and fascinating road to be travelled before the many roles of trace elements in mammalian metabolism are fully appreciated.

REFERENCES

Allen, K. G. D., Arthur, J. R., Morrice, P. C., Nicol, F. & Mills, C. F. (1988). Copper deficiency and tissue glutathione concentrations in the rat. *Proceedings of the Society for Experimental Biology and Medicine* **187**, 38–43.

Arias, I. M., Fleischer, G., Kirsch, R., Mishkin, S. & Gatmaintan, Z. (1976). On the structure and function of ligandin. In *Glutathione: Metabolism and Function, Kroc Foundation Series* vol. 6, pp. 175–188 [I. M. Arias and W. B. Jakoby, editors]. New York, Raven Press.

Arthur, J. R. (1988). Effects of selenium and vitamin E status on plasma creatine kinase activity in calves. *Journal of Nutrition* **118**, 747–755.

Arthur, J. R. & Boyne, R. (1985). Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. *Life Sciences* **36**, 1569–1575.

Arthur, J. R., Boyne, R., Hill, H. A. O. & Okolow-Zubkowska, M. J. (1981). The production of oxygen-derived radicals by neutrophils from selenium-deficient cattle. *FEBS Letters* **135**, 187–190.

Arthur, J. R., McPhail, D. B. & Goodman, B. A. (1988*c*). Spin trapping of free radicals in homogenates of heart from selenium and vitamin E deficient rats. *Free Radical Research Communications* **4**, 311–315.

Arthur, J. R., Morrice, P. C. & Beckett, G. J. (1988*a*). Thyroid hormone concentrations in selenium deficient and selenium-sufficient cattle. *Research in Veterinary Science* **45**, 122–123.

Arthur, J. R., Morrice, P. C., Nicol, F., Beddows, S. E., Boyd, R., Hayes, J. D. & Beckett, G. J. (1987). The effects

of selenium and copper deficiencies on glutathione S-transferase and glutathione peroxidase in rat liver. *Biochemical Journal* **248**, 539–544.

Arthur, J. R., Nicol, F., Boyne, R., Allen, K. G. D., Hayes, J. D. & Beckett, G. J. (1988*b*). Old and new roles for selenium. In *Trace Substances in Environmental Health*, vol. **xxi**, pp. 487–498 [D. D. Hemphill, editor]. University of Missouri.

Asayama, K., Kooy, N. W. & Burr, I. M. (1986). Effect of vitamin E deficiency and selenium deficiency on insulin secretory reserve and free radical scavenging systems in islets: decrease of islet manganese superoxide dismutase. *Journal of Laboratory and Clinical Medicine* **107**, 459–464.

Baker, S. S. & Cohen, H. J. (1984). Increased sensitivity to H_2O_2 in glutathione peroxidase-deficient rat granulocytes. *Journal of Nutrition* **114**, 2003–2009.

Bales, C. W., Wang, M. C., Freeland-Graves, J. H. & Pobocik, R. S. (1986). The effects of Zn deficiency and food restriction on PGE_2 and thromboxane B_2 in saliva and plasma of rats. *Prostaglandins* **31**, 859–868.

Baly, D. L., Curry, D. L., Keen, C. L. & Hurley, L. S. (1984). Effect of manganese on insulin secretion and carbohydrate homeostasis in rats. *Journal of Nutrition* **114**, 1438–1446.

Bambara, R. A., Crute, J. J. & Wahl, A. F. (1985). Is Ap_4A an activator of eukaryotic DNA replication. *Cancer Investigation* **3**, 473–479.

Baril, E. P., Coughlin, S. A. & Zamecnik, P. C. (1985). 5'5'''P^1P^4 diadenosine tetraphosphate (Ap_4 A): a putative initiator of DNA replication. *Cancer Investigation* **3**, 465–471.

Beard, J., Green, W., Miller, L. & Finch, C. (1984). Effects of Fe-deficiency anaemia on hormone levels and thermogenesis during cold exposure. *American Journal of Physiology* **247**, R114–R119.

Beckett, G. J., Beddows, S. E., Morrice, P. C., Nicol, F. & Arthur, J. R. (1987). Inhibition of hepatic deiodination of thyroxine caused by selenium deficiency in rats. *Biochemical Journal* **248**, 443–447.

Blanquet, S., Plateau, P. & Brevet, A. (1983). The role of Zn in 5',5' diadenosine tetraphosphate production by aminoacyl-tRNA synthetases. *Molecular and Cellular Biochemistry* **52**, 3–11.

Bolze, M. S., Reeves, R. D., Lindbeck, F. E. & Elders, M. J. (1987). Influence of zinc on growth, somatomedin, and glycosaminoglycan metabolism in rats. *American Journal of Physiology* **252**, E21–E26.

Bolze, M. S., Reeves, R. D., Lindbeck, F. E., Kemp, S. F. & Elders, M. J. (1985). Influence of Mn on growth, somatomedin and glycosaminoglycan metabolism. *Journal of Nutrition* **115**, 352–358.

Boyne, R. & Arthur, J. R. (1979). Alterations in neutrophil function in selenium-deficient cattle. *Journal of Comparative Pathology* **89**, 151–158.

Boyne, R. & Arthur, J. R. (1981). Effect of selenium and copper deficiency on neutrophil function in cattle. *Journal of Comparative Pathology* **91**, 271–276.

Boyne, R. & Arthur, J. R. (1986*a*). The response of selenium deficient mice to *Candida albicans* infection. *Journal of Nutrition* **116**, 816–822.

Boyne, R. & Arthur, J. R. (1986*b*). Effects of molybdenum or iron induced copper deficiency on the viability and function of neutrophils from cattle. *Research in Veterinary Science* **41**, 417–419.

Boyne, R., Arthur, J. R. & Wilson, A. B. (1986). An *in vivo* and *in vitro* study of selenium deficiency and infection in rats. *Journal of Comparative Pathology* **96**, 379–386.

Boyne, R., Mann, S. O. & Arthur, J. R. (1984). Effect of *Salmonella typhimurium* infection on selenium-deficient rats. *Microbios Letters* **27**, 83–87.

Bray, T. M., Kubow, S. & Bettger, W. J. (1986). Effect of dietary Zn on endogenous free radical production in rat lung microsomes. *Journal of Nutrition* **116**, 1054–1060.

Bremner, I., Morrison, J. N., Wood, A. M. & Arthur, J. R. (1987). 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.

Bryant, R. W., Simon, T. C. & Bailey, J. M. (1983). Hydroperoxy fatty acid formation in selenium deficient rat platelets: coupling of glutathione peroxidase to the lipoxygenase pathway. *Biochemical and Biophysical Research Communications* **117**, 183–189.

Bunk, M. J. & Combs, G. F. (1981). Evidence for impaired conversion of methionine to cysteine in the selenium-deficient chick. *Proceedings of the Society for Experimental Biology and Medicine* **167**, 87–93.

Burk, R. F. (1983). Biological activity of selenium. *Annual Review of Nutrition* **3**, 53–70.

Burk, R. F. & Gregory, P. E. (1982). Some characteristics of ^{75}Se-P a selenoprotein found in rat liver and plasma, and comparison of it with selenoglutathione peroxidase. *Archives of Biochemistry and Biophysics* **213**, 73–80.

Chambers, I., Frampton, J., Goldfarb, P., Affara, N., Bain, W. & Harrison, P. R. (1986). The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the termination codon, TGA. *EMBO Journal* **5**, 1221–1227.

Chen, S.-Y. (1986). Autoradiographic study of cell proliferation in acanthotic buccal epithelium of Zn-deficient rabbits. *Archives of Oral Biology* **31**, 535–539.

Chesters, J. K. (1978). Biochemical functions of zinc in animals. *World Review of Nutrition and Dietetics* **32**, 135–164.

Chesters, J. K. & Quarterman, J. (1970). Effects of zinc deficiency on food intake and feeding patterns of rats. *British Journal of Nutrition* **24**, 1061–1069.

Chesters, J. K. & Will, M. (1981). Measurement of zinc flux through the plasma in normal and endotoxin-stressed pigs and the effects of Zn supplementation during stress. *British Journal of Nutrition* **46**, 119–130.

Combs, G. F. & Combs, S. B. (1986). *The Role of Selenium in Nutrition.* New York: Academic Press Inc.

Cossack, Z. T. (1986). Somatomedin-C and zinc status in rats as affected by Zn, protein and food intake. *British Journal of Nutrition* **56**, 163–169.

Cowen, L. A., Bell, D. E., Hoadley, J. E. & Cousins, R. J. (1986). Influence of dietary zinc deficiency and parenteral zinc on the rat liver fructose 1,6 bisphosphatase activity. *Biochemical and Biophysical Research Communications* **134**, 944–950.

Crossley, L. G., Falchuk, K. H. & Vallee, B. L. (1982). Messenger ribonucleic acid function and protein synthesis in Zn-deficient *E. gracilis. Biochemistry* **21**, 5359–5363.

Dallman, P. R. (1986). Biochemical basis for the manifestations of Fe deficiency. *Annual Review of Nutrition* **6**, 13–40.

Dallman, P. R. (1987). Iron deficiency and the immune response. *American Journal of Clinical Nutrition* **46**, 329–334.

Dallman, P. R., Refino, C. & Yland, M. J. (1982). Sequence of development of Fe deficiency in the rat. *American Journal of Clinical Nutrition* **35**, 671–677.

Dardenne, M., Pleau, J.-M., Nabarra, B., Lefrancier, P., Derrien, M., Choay, J. & Bach, J.-F. (1982). Contribution of Zn and other metals to the biological activity of the serum thymic factor. *Proceedings of the National Academy of Sciences of the U.S.A.* **79**, 5370–5373.

Dardenne, M., Savino, W., Wade, S., Kaiserlian, D., Lemonnier D. & Bach, J.-F. (1984). *In vivo* and *in vitro* studies of thymulin in marginally Zn-deficient mice. *European Journal of Immunology* **14**, 454–458.

Davies, K. J. A., Donovan, C. M., Refino, C. J., Brooks, G. A., Packer, L. & Dallman, P. R. (1984). Distinguishing effects of anaemia and muscle Fe deficiency on exercise bioenergetics in the rat. *American Journal of Physiology* **246**, E535–E543.

Davies, N. T. & Lawrence, C. B. (1986). Studies on the effects of copper deficiency on rat liver mitochondria. III. Effects on adenine nucleotide translocase. *Biochimica et Biophysica Acta* **848**, 294–304.

Davies, N. T., Lawrence, C. B. & Mills, C. F. (1985). Studies on the effects of copper deficiency on rat liver mitochondria II. Effects on oxidative phosphorylation. *Biochimica et Biophysica Acta* **809**, 362–368.

De Pasquale-Jardieu, P. & Fraker, P. J. (1980). Further characterization of the role of corticosterone in the loss of humoral immunity in the Zn-deficient A/J mouse as determined by adrenalectomy. *Journal of Immunology* **124**, 2650–2655.

Dougherty, J. J., Croft, W. A. & Hoekstra, W. G. (1981). Effects of ferrous chloride and iron-dextran on lipid peroxidation *in vivo* in vitamin E and selenium adequate and deficient rats. *Journal of Nutrition* **111**, 1784–1796.

Dowd, P. S., Kelleher, J. & Guillou, P. J. (1986). T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats. *British Journal of Nutrition* **55**, 59–69.

Dreosti, I. E., Record, I. & Manuel, S. J. (1985). Zinc deficiency and the developing embryo. *Biological Trace Element Research* **7**, 103–122.

Eskew, M. L., Scholz, R. W., Reddy, C. C., Todhunter, D. A. & Zarkower, A. (1985). Effects of vitamin E and selenium deficiencies on rat immune function. *Immunology* **54**, 173–180.

Evans, T. C. & Mackler, B. (1985). Effects of Fe deficiency on energy conservation in rat liver and skeletal muscle submitochondrial particles. *Biochemical Medicine* **34**, 93–99.

Evenson, J. K. & Sunde, R. A. (1988). Selenium incorporation into seleno-proteins in the Se-adequate and Se-deficient rat. *Proceedings of the Society for Experimental Biology and Medicine* **187**, 169–180.

Fell, B. F. (1981). Pathological consequences of copper deficiency and cobalt deficiency. *Philosophical Transactions of The Royal Society* London **294B**, 153–169.

Fell, B. F. (1987). The pathology of copper deficiency in animals. *In Copper in Animals and Man*, vol. 2, pp. 1–28 [J. McC. Howell and J. N. Gawthorne, editors]. Boca Raton, Florida: CRC Press.

Fell, B. F., Farmer, L. J., Farquharson, C., Bremner, I. & Graca, D. S. (1985). Observations on the pancreas of cattle deficient in copper. *Journal of Comparative Pathology* **95**, 573–590.

Fields, M., Ferretti, R. J., Smith, J. C. & Reiser, S. (1984*a*). Effect of dietary carbohydrate and copper status on blood pressure of rats. *Life Sciences* **34**, 763–769.

Fields, M., Ferretti, R. J., Smith, J. C. & Reisner, S. (1984*b*). Interaction between dietary carbohydrate and copper nutriture on lipid peroxidation in rat tissues. *Biological Trace Element Research* **6**, 379–391.

Fogerty, A. C., Ford, G. L., Dreosti, I. E. & Tinsley, I. J. (1985). Zn deficiency and fatty acid composition of tissue lipids. *Nutrition Reports International* **32**, 1009–1019.

Fraga, C. G., Arias, R. F., Llesuy, S. F., Koch, O. R. & Boveris, A. (1987). Effect of vitamin E- and selenium-deficiency on rat liver chemiluminescence. *Biochemical Journal* **242**, 383–386.

Fraker, P. J., Gershwin, M. E., Good, R. A. & Prasad, A. S. (1986). Inter-relations between Zn and immune function. *Federation Proceedings* **45**, 1474–1479.

Fraker, P. J., Hildebrandt, K. & Luecke, R. W. (1984). Alteration of antibody-mediated responses of suckling mice to T-cell dependent and independent antigens by maternal marginal Zn deficiency, restoration of responsivity by nutritional repletion. *Journal of Nutrition* **114**, 170–179.

Funk, C. D., Boubez, W. & Powell, W. S. (1987). Effects of selenium-deficient diets on the production of prostaglandins and other oxygenated metabolites of arachidonic acid and linoleic acid by rat and rabbit aortae. *Biochimica et Biophysica Acta* **921**, 213–220.

Gerson, S. J., Meyer, J. & Gandor, D. (1983). Decreased Zn concentration does not lead to atrophy of rat oral epithelium. *Journal of Nutrition* **113**, 820–823.

Giugliano, R. & Millward, D. J. (1987). The effects of severe zinc deficiency on protein turnover in muscle and thymus. *British Journal of Nutrition* **57**, 139–155.

Goldblum, S. E., Cohen, D. A., Jay, M. & McClain, C. J. (1987). Interleukin 1-induced depression of iron and zinc: role of granulocytes and lactoferrin. *American Journal of Physiology* **252**, E27–E32.

Gordon, P. R., Browning, J. D. & O'Dell, B. L. (1983). Platelet arachidonic acid metabolism and platelet function in Zn-deficient rats. *Journal of Nutrition* **113**, 766–772.

Grossman, A. & Wendel, A. (1983). Non-reactivity of the selenoenzyme glutathione peroxidase with enzymically hydroperoxidised phospholipids. *European Journal of Biochemistry* **135**, 549–552.

Grummt, F., Weinmann-Dorsch, C., Schneider-Schaulies, J. & Lux, A. (1986). Zinc as a second messenger of mitogenic induction. *Experimental Cell Research* **163**, 191–200.

Hafeman, D. G., Sunde, R. A. & Hoekstra, W. G. (1974). The effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *Journal of Nutrition* **104**, 580–587.

Hammermueller, J. D., Bray, T. M. & Bettger, W. J. (1987). Effects of zinc and copper deficiencies on microsomal NADPH-dependent active oxygen generation in rat lung and liver. *Journal of Nutrition* **117**, 894–901.

Hanas, J. S., Hazuda, D. J., Bogenhagen, D. F., Wu, F. Y.-H. & Wu, C.-W. (1983). *Xenopus* transcription factor A requires Zn for binding to the 5S RNA gene. *Journal of Biological Chemistry* **258**, 14120–14125.

Haplin, K. M. & Baker, D. H. (1984). Selenium deficiency and trans-sulfuration in the chick. *Journal of Nutrition* **114**, 606–612.

Helyar, L. & Sherman, A. R. (1987). Iron deficiency and interleukin-I production by rat leukocytes. *American Journal of Clinical Nutrition* **46**, 346–352.

Hicks, S. E. & Wallwork, J. C. (1987). Effect of dietary Zn deficiency on protein synthesis in cell-free systems isolated from rat liver. *Journal of Nutrition* **117**, 1234–1240.

Hill, K. E. & Burk, R. F. (1985). Effect of selenium deficiency on the disposition of plasma glutathione. *Archives of Biochemistry and Biophysics* **240**, 166–171.

Hill, K. E., Burk, R. F. & Lane, J. M. (1987). Effect of selenium depletion and repletion on plasma glutathione and glutathione dependent enzymes in the rat. *Journal of Nutrition* **117**, 99–104.

Hunsaker, H. A., Morita, M. & Allen, K. G. D. (1984). Marginal copper deficiency in rats. Aortal morphology and cholesterol values in first generation adult males. *Atherosclerosis* **51**, 1–19.

Hurley, L. S. (1981). Teratogenic aspects of Mn, Zn & Cu nutrition. *Physiological Reviews* **61**, 249–295.

Hwang, D. H., Chanmugan, P. & Wheeler, C. (1984). Zinc deficiency affects neither platelet arachidonic acid nor platelet aggregation in rats. *Journal of Nutrition* **114**, 398–403.

Jenkinson, S. G., Lawrence, R. A., Burk, R. F. & Williams, D. M. (1982). Effects of copper deficiency on the activity of the selenoenzyme glutathione peroxidase and on excretion and tissue retention of $^{75}SeO_3^{2-}$. *Journal of Nutrition* **112**, 197–204.

Jones, D. G. & Suttle, N. F. (1981). Some effects of copper deficiency on leucocyte function in sheep and cattle. *Research in Veterinary Science* **31**, 151–156.

Jones, D. G. & Suttle, N. F. (1983). The effect of copper deficiency on the resistance of mice to infection with *Pasteurella haemolytica*. *Journal of Comparative Pathology* **93**, 143–149.

Joseph, C. E., Ashrafi, S. H. & Waterhouse, J. P. (1981). Structural changes in rabbit oral epithelium caused by Zn deficiency. *Journal of Nutrition* **111**, 53–57.

Kasarskis, E. J., Sparks, D. L. & Slevin, J. T. (1986). Changes in hypothalamic noradrenergic systems during the anorexia of Zn deficiency. *Biological Trace Element Research* **9**, 25–35.

Kennedy, S., Rice, D. A. & Davidson, W. B. (1987). Experimental myopathy in vitamin E and selenium depleted calves with and without added dietary poly-unsaturated fatty acid as a model for nutritional degenerative myopathy in ruminant cattle. *Research in Veterinary Science* **43**, 384–394.

Kirchgessner, M. & Roth, H.-P. (1975). Estimation of metabolic availability of zinc and assessment of zinc requirements from changes in zinc-metalloenzymes. *Archiv für Tierernährung* **25**, 83–92.

Klug, A. & Rhodes, D. (1987). Zinc fingers: a novel protein motif for nucleic acid recognition. *Trends in Biochemical Sciences* **12**, 464–469.

Knight, S. A. & Sunde, R. A. (1987). The effect of progressive selenium deficiency on anti-glutathione peroxidase antibody reactive protein in rat liver. *Journal of Nutrition* **117**, 732–738.

Koller, L. D., Mulhern, S. A., Frankel, N. C., Steven, M. G. & Williams, J. R. (1987). Immune dysfunction in rats fed a diet deficient in copper. *American Journal of Clinical Nutrition* **45**, 997–1006.

Kopp, S., Klevay, L. M. & Feliksik, J. M. (1983). Physiological and metabolic characterization of a cardiomyopathy induced by chronic copper deficiency. *American Journal of Physiology* **245**, H855–H866.

Korte, J. J. & Prohaska, J. R. (1987). Dietary copper deficiency alters protein and lipid composition of murine lymphocyte plasma membranes. *Journal of Nutrition* **117**, 1076–1084.

Kramer, T. R., Briske-Anderson, M., Johnson, S. B. & Holman, R. T. (1984). Influence of reduced food intake on polyunsaturated fatty acid metabolism in Zn-deficient rats. *Journal of Nutrition* **114**, 1224–1230.

Kuvibidila, S. (1987). Iron deficiency, cell mediated immunity and resistance to infections: present knowledge and controversies. *Nutrition Research* **7**, 989–1003.

Lawrence, C. B., Davies, N. T., Mills, C. F. & Nicol, F. (1985). Studies on the effects of copper deficiency on rat liver mitochondria. I. Changes in mitochondrial composition. *Biochimica et Biophysica Acta* **809**, 351–361.

Li, E. T. S. & O'Dell, B. L. (1986). Effect of Zn status on the binding of prostaglandins to ovarian membranes and intact platelets of pregnant rats. *Journal of Nutrition* **116**, 1448–1455.

Lukasewycz, O. A. & Prohaska, J. R. (1983). Lymphocytes from copper-deficient mice exhibit decreased mitogen reactivity. *Nutrition Research* **3**, 335–341.

Lytton, F. D. C. & Bunce, G. E. (1986). Dietary zinc and parturition in the rat. 1. Uterine pressure cycles. *Biological Trace Element Research* **9**, 151–163.

McClain, C. J., Kasarskis, E. J. & Allen, J. J. (1985). Functional consequences of Zn deficiency. *Progress in Food and Nutrition Science* **9**, 185–226.

Magneson, G. R., Puvathingal, J. M. & Ray, W. J. (1987). The concentrations of free Mg^{2+} and free Zn^{2+} in equine blood plasma. *Journal of Biological Chemistry* **262**, 1140–1148.

Masters, D. G., Keen, C. L., Lonnerdal, B. & Hurley, L. S. (1983). Zn deficiency teratogenicity, the protective role of maternal tissue catabolism. *Journal of Nutrition* **113**, 905–912.

Masters, D. G., Keen, C. L., Lonnerdal, B. & Hurley, L. S. (1986). Release of zinc from maternal tissue during Zn deficiency or simultaneous Zn and Ca deficiency in the pregnant rat. *Journal of Nutrition* **116**, 2148–2154.

Masukawa, T., Goto, J. & Iwata, H. (1983). Impaired metabolism of arachidonate in selenium-deficient animals. *Experientia* **39**, 405–406.

May, P. M., Linder, P. W. & Williams, D. R. (1977). Computer simulation of metal ion equilibria in biofluids: Models for the low molecular weight distribution of Ca(II), Mg(II), Mn(II), Fe(II), Cu(II), Zn(II), and Pb(II) ions in human blood plasma. *Journal of the Chemical Society. Dalton Transactions*, 588–595.

Mercalli, M. E., Seri, S., Aquilio, E., Cramarossa, L., Del Gobbo, V., Accinni, L. & Toniette, G. (1984). Zinc deficiency and thymus ultrastructure in rats. *Nutrition Research* **4**, 665–671.

Miller, J., McLachlan, A. D. & Klug, A. (1985). Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. *EMBO Journal* **4**, 1609–1614.

Motsenbocker, M. A. & Tappel, A. L. (1984). Effect of dietary selenium on plasma selenoprotein P, selenoprotein P1 and glutathione peroxidase in the rat. *Journal of Nutrition* **114**, 279–285.

Murkawa, H., Bland, C. E., Willis, W. T. & Dallman, P. R. (1987). Iron deficiency and neutrophil function: different rates of correction of the depression in oxidative burst and myeloperoxidase activity after Fe treatment. *Blood* **69**, 1464–1468.

Murray, J. M. & Murray, A. B. (1985). The effects of selenium deficiency and repletion in the host resistance to infection. In *Trace Element Metabolism in Man and Animals-5*, pp. 244–247 [C. F. Mills, I. Bremner and J. K. Chesters, editors]. Slough: CAB.

Ng, W. L., Fong, L. Y. Y., Ma, L. & Newberne, P. M. (1984). Dietary Zn deficiency and tumorigenesis, a scanning EM study, *Journal of Electron Microscopy* **33**, 344–348.

Nielsen, F. H., Hunt, C. D., Mullen, L. M. & Hunt, J. R. (1987). Effect of dietary boron on mineral, estrogen and testosterone metabolism in postmenopausal women. *FASEB Journal* **1**, 394–397.

Oner, G., Bhaumick, B. & Bala, R. M. (1984). Effect of Zn deficiency on serum somatomedin levels and skeletal growth in young rats. *Endocrinology* **114**, 1860–1863.

Orgebin-Crist, M. C., Freeman, M. & Barney, G. H. (1971). Sperm formation in Zn-deficient rats. *Annales de Biologie Animale, Biochimie, Biophysique* **11**, 547–558.

Ortega, S. S., Cachaza, J. A., Tovar, I. V. & Feijoo, M. F. (1985). Zinc deficiency dermatitis in parenteral nutrition: an electron microscopic study. *Dermatologica* **171**, 163–169.

Otter, R., Goldberg, M., Vogl, S. & Wendel, A. (1986). Increased proliferative activity in selenium-deficient mouse liver. *Chemico-Biological Interactions* **52**, 295–300.

Park, J. H. Y., Grandjean, C. J., Antonson, D. L. & Vanderhoof, J. A. (1985). Effects of short-term isolated Zn deficiency on intestinal growth and activities of several brush border enzymes in weanling rats. *Pediatric Research* **19**, 1333–1336.

Parnham, M. J., Winkelmann, J. & Leyck, S. (1983). Macrophage, lymphocyte and chronic inflammatory responses in selenium deficient rodents. Association with decreased glutathione peroxidase activity. *International Journal of Immunopharmacology* **5**, 455–461.

Pascoe, G. A., Sakai Wong, J., Soliven, E. & Correia, M. A. (1983). Regulation of intestinal cytochrome P-450 and heme by dietary nutrients. Critical role of selenium. *Biochemical Pharmacology* **32**, 3027–3035.

Paynter, D. I. (1980*a*). Changes in activity of the manganese superoxide dismutase enzyme in tissues of rats with changes in dietary manganese. *Journal of Nutrition* **110**, 437–447.

Paynter, D. I. (1980*b*). The role of dietary copper, manganese, selenium and vitamin E in lipid peroxidation in tissues of the rat. *Biological Trace Element Research* **2**, 121–135.

Paynter, D. I. & Martin, G. B. (1980). Investigations into combined dietary deficiencies of copper, selenium and vitamin E in the rat. *Biological Trace Element Research* **2**, 175–191.

Paynter, D. I., Moir, R. J. & Underwood, E. J. (1979). Changes in the activity of the Cu-Zn superoxide dismutase enzyme in tissues of the rat with changes in dietary copper. *Journal of Nutrition* **109**, 1570–1576.

Perkkio, M. V., Jansson, L. T., Dallman, P. R., Siimes, M. A. & Savilahti, E. (1987). sIgA and IgM-containing cells in the intestinal mucosa of iron-deficient rats. *American Journal of Clinical Nutrition* **46**, 341–345.

Peter, F. & Wang, S. (1981). Serum iron and total iron binding capacity compared with serum ferritin in assessment of iron deficiency. *Clinical Chemistry* **27**, 276–279.

Prasad, A. S. (ed.) (1982). Clinical and biochemical spectrum of zinc deficiency in human subjects. In *Clinical, Biochemical and Nutritional Aspects of Trace Elements*, pp. 3–62. New York: A. R. Liss.

Prasad, A. S., Oberleas, D., Wolff, P. & Horwitz, J. P. (1969). Effect of growth hormone on non-hypophysectomized zinc-deficient rats and zinc on hypophysectomized rats. *Journal of Laboratory and Clinical Medicine* **73**, 486–494.

Prohaska, J. R. & Cox, D. A. (1983). Decreased brain ascorbate levels in copper-deficient mice and in brindled mice. *Journal of Nutrition* **113**, 2623–2629.

Prohaska, J. R. & Heller, L. J. (1982). Mechanical properties of the copper-deficient rat heart. *Journal of Nutrition* **112**, 2142–2150.

Prohaska, J. R. & Lukasewycz, O. A. (1981). Copper deficiency suppresses the immune response in mice. *Science* **213**, 559–561.

Quisumbing, T. L., Wong, T. M., Jen, L. S. & Loh, T. T. (1985). Biochemical effects of mild iron deficiency and cold acclimatisation on rat skeletal muscle. *Biochemical Medicine* **34**, 355–363.

Record, I. R., Dreosti, I. E., Tulsi, R. S. & Manuel, S. J. (1986). Maternal metabolism and teratogenicity in Zn-deficient rats. *Teratology* **33**, 311–317.

Reeves, P. G. & O'Dell, B. L. (1986). Effects of dietary zinc deprivation on the activity of angiotensin-converting enzyme in serum of rats and guinea pigs. *Journal of Nutrition* **116**, 128–134.

Reiser, S., Ferretti, R. J., Fields, M. & Smith, J. C. (1983). Role of dietary fructose in the enhancement of mortality and biochemical changes associated with copper deficiency in rats. *American Journal of Clinical Nutrition* **38**, 214–222.

Reiter, R. & Wendel, A. (1983). Selenium and drug metabolism-I, Multiple modulations of mouse liver enzymes. *Biochemical Pharmacology* **32**, 3063–3067.

Reiter, R. & Wendel, A. (1984). Selenium and drug metabolism-II, Independence of glutathione peroxidase and reversibility of hepatic enzyme modulations in deficient mice. *Biochemical Pharmacology* **33**, 1923–1928.

Reiter, R. & Wendel, A. (1985). Selenium and drug metabolism-III, Relation of glutathione peroxidase and other hepatic enzyme modulations to dietary supplements. *Biochemical Pharmacology* **34**, 2287–2290.

Robinson, M. F. & Thomson, C. D. (1983). The role of selenium in the diet. *Nutrition Abstracts and Reviews* **53**, 3–26.

Samman, S. & Roberts, D. C. K. (1985). Dietary copper and cholesterol metabolism. *Nutrition Research* **5**, 1021–1034.

Serfass, R. E. & Ganther, H. E. (1975). Defective microbicidal killing in glutathione peroxidase-deficient rats. *Nature* **255**, 640–641.

Siddons, R. C. & Mills, C. F. (1981). Glutathione peroxidase activity and erythrocyte stability in calves differing in selenium and vitamin E status. *British Journal of Nutrition* **46**, 345–355.

Southon, S., Livesey, G., Gee, J. M. & Johnson, I. T. (1985). Intestinal cell proliferation and protein synthesis in zinc-deficient rats. *British Journal of Nutrition* **53**, 595–603.

Spears, J. W. (1984). Nickel as a 'newer trace element' in the nutrition of domestic animals. *Journal of Animal Science* **59**, 823–835.

Sullivan, J. F., Jetton, M. M., Hahn, H. K. J. & Burch, R. E. (1980). Enhanced lipid peroxidation in liver microsomes of Zn-deficient rats. *American Journal of Clinical Nutrition* **33**, 51–56.

Sunde, R. A. (1984). The biochemistry of selenoproteins. *Journal of the American Oil Chemists Society* **61**, 1891–1900.

Sunde, R. A. & Evenson, J. K. (1987). Serine incorporation into the selenocysteine moiety of glutathione peroxidase. *Journal of Biological Chemistry* **262**, 933–937.

Takahashi, K. & Cohen, H. J. (1986). Selenium-dependent glutathione peroxidase protein and activity: Immunological investigations on cellular and plasma enzymes. *Blood* **68**, 640–645.

Takahashi, K., Newburger, P. E. & Cohen, H. J. (1986). Glutathione peroxidase protein: Absence in selenium deficiency states and correlation with ezymatic activity. *Journal of Clinical Investigation* **77**, 1402–1404.

Underwood, E. J. & Somers, M. (1969). Studies of zinc nutrition in sheep. 1. The relation of zinc to growth, testicular development and spermatogenesis in young rams. *Australian Journal of Agricultural Research* **20**, 889–897.

Ursini, F., Maiorino, M. & Gregolin, C. (1985). The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochimica et Biophysica Acta* **839**, 62–70.

Vallee, B. L. & Galdes, A. (1984). The metallobiochemistry of Zn enzymes. *Advances in Enzymology* **56**, 283–430.

Wallwork, J. C., Fosmire, G. J. & Sandstead, H. H. (1981). Effect of zinc deficiency on appetite and plasma amino acid concentrations in the rat. *British Journal of Nutrition* **45**, 127–136.

Walsh, C. T. & Orme-Johnson, W. H. (1987). Nickel enzymes. *Biochemistry* **26**, 4901–4906.

Weinberg, E. D. (1984). Iron withholding: a defense against infection and neoplasia. *Physiological Reviews* **64**, 65–102.

Wendel, A. & Otter, R. (1987). Alterations in the intermediary metabolism of selenium-deficient mice. *Biochimica et Biophysica Acta* **925**, 94–100.

Williams, R. B. & Chesters, J. K. (1970). The effects of early zinc deficiency on DNA and protein synthesis in the rat. *British Journal of Nutrition* **24**, 1053–1059.

Williams, R. B. & Mills, C. F. (1970). The experimental production of zinc deficiency in the rat. *British Journal of Nutrition* **24**, 989–1003.

Wingender, E., Dilloo, D. & Seifert, K. H. (1984). Zinc ions are differentially required for the transcription of ribosomal 5S RNA and tRNA in a HeLa cell extract. *Nucleic Acid Research* **12**, 8971–8985.

Yang, J.-G., Morrison-Plummer, J. & Burk, R. F. (1987). Purification and quantitation of a rat plasma selenoprotein distinct from glutathione peroxidase using monoclonal antibodies. *Journal of Biological Chemistry* **262**, 13372–13375.

Zidenberg-Cherr, S., Keen, C. L., Lonnerdal, B. & Hurley, L. S. (1983). Superoxide dismutase activities and lipid peroxidation in the rat: developmental correlation affected by Mn deficiency. *Journal of Nutrition* **113**, 2498–2504.