BIOLOGY OF ZINC AND BIOLOGICAL VALUE OF DIETARY ORGANIC ZINC COMPLEXES AND CHELATES

JOHANNES W. G. M. SWINKELS[123], ERVIN T. KORNEGAY[1] AND MARTIN W. A. VERSTEGEN[4]

[1] Department of Animal Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[2] Current address: Research Institute for Pig Husbandry, P.O. Box 83, 5240 AB Rosmalen, The Netherlands
[3] The author was a Pratt Fellow and this research was supported by the John Lee Pratt Animal Nutrition Foundation
[4] Department of Animal Nutrition, Agricultural University of Wageningen, Haagsteeg 4, 6708 PM Wageningen, The Netherlands

CONTENTS

INTRODUCTION 130
BIOLOGY OF ZINC 130
ZINC IN PORCINE TISSUES AND FLUIDS 130
INTRACELLULAR DISTRIBUTION OF ZINC 130
BIOLOGICAL FUNCTIONS OF INTRACELLULAR ZINC 131
ZINC HOMEOSTASIS 133
ASSESSMENT OF ZINC STATUS 133
BIOLOGICAL ADAPTATIONS DURING ZINC DEFICIENCY 135
ABSORPTION OF ZINC 136
ROLE OF INTESTINAL METALLOTHIONEIN IN ZINC ABSORPTION . . . 136
SITE(S) OF ZINC ABSORPTION 137
MINERALS INTERACTING WITH ZINC DURING ZINC ABSORPTION . . 139
INTRINSIC FACTORS AFFECTING ZINC ABSORPTION 140
BIOLOGICAL VALUE OF DIETARY COMPLEXES AND CHELATES OF ZINC 140
METAL COMPLEXES AND CHELATES 140
CONSIDERATIONS WHEN DETERMINING BIOAVAILABILITY OF ZINC . . 141
AVAILABILITY OF ZINC COMPLEXED WITH PICOLINIC ACID 142
AVAILABILITY OF ZINC COMPLEXED WITH METHIONINE OR OTHER AMINO ACIDS 142
CONCLUSIONS 145
REFERENCES 145

INTRODUCTION

Growth retardation and an abnormal hair coat, induced in rats by feeding a purified diet low in Zn (1·6 p.p.m. Zn), were the first clinical signs associated with dietary Zn deficiency (Todd *et al.* 1934). Two decades later, a direct relationship between Zn and growth was reported for swine (Tucker & Salmon, 1955). In addition to impaired growth in the Zn deficient pigs, Tucker & Salmon (1955) also observed dermatosis that was previously called 'parakeratosis' by Kernkamp & Ferrin in 1953. Parakeratosis is the classic characteristic associated with severe Zn deficiency in swine (National Research Council, 1979).

Since the recognition of Zn as an essential nutrient, many researchers have studied the role of Zn in biology and nutrition. In the first section of this paper the biology of Zn is reviewed. This is followed by an overview of our current understanding of possible modes of action of Zn absorption. In the last part the biological value of dietary organic Zn complexes and Zn chelates is discussed.

BIOLOGY OF ZINC

ZINC IN PORCINE TISSUES AND FLUIDS

The concentration of Zn in the whole body of the pig, expressed on a fat free basis, is 25 mg/kg (Spray & Widdowson, 1950). This is within the 20–30 mg/kg range reported for fat free bodies of rats, cats, man (Spray & Widdowson, 1950), sheep (Grace, 1983) and dairy cows (Miller *et al.* 1974). In Table 1, concentrations of Zn in tissues, fluids, bone and integuments of the pig are presented. These concentrations were similar to those found in body compartments of rats, sheep, cows, monkeys and men (Hambidge *et al.* 1986; Jackson, 1989).

In pigs, the highest concentration of Zn is found in hair. However, relative to the Zn content of the whole body the total amount of Zn in hair is small. The largest pool of Zn, approximately 60%, is found in skeletal muscle tissue because of its bulk and fairly high Zn concentration (Jackson, 1989). The concentration of Zn varies with the type of skeletal muscle, being highest in red and lowest in white skeletal muscle (Cassens *et al.* 1967). The remainder of the Zn pool of the body is primarily located in bone and organs. Body fluids contain only a small proportion of total body Zn (Hambidge *et al.* 1986).

The total Zn content of the body on a weight basis remains fairly constant from birth to maturity (Spray & Widdowson, 1950). The relative proportion of body Zn found in the liver gradually increases from birth to weaning. After weaning, the liver Zn concentration rapidly decreases to approximately the level of Zn present at birth (Spray & Widdowson, 1950).

INTRACELLULAR DISTRIBUTION OF ZINC

In mouse liver cells, the largest proportion of intracellular Zn was found in the light fraction containing organelles other than mitochondria and nuclei (Bartholomew *et al.* 1959). Conversely, in porcine muscle cells the largest concentration of Zn was found in the heavy fraction containing myofibrils and nuclei (Cassens *et al.* 1967). Moreover, it was found that the level of Zn in the heavy fraction of red skeletal muscle was almost four times as high as in white skeletal muscle. The Zn level in the light fraction was equally low for both the red and white muscle types (Cassens *et al.* 1967). Thus, the intracellular distribution of Zn varies among tissues.

It is much harder to determine the nature of intracellular Zn than its location. Most biochemical techniques currently available involve destruction of the cell allowing Zn to

Table 1. *Concentration of Zn in body fluids and tissues of fast growing pigs*[a]

Item	Concentration
Blood	
Plasma (μg/l)	740
Serum (μg/l)	600
Erythrocytes (μg/g packed cells)	7·7
Leucocytes[b]	21·5
Tissues[b]	
Bone	113
Brain	70
Heart	96
Kidney	141
Liver	151
Red muscle	137
Mixed muscle	89
White muscle	67
Pancreas	161
Spleen	107
Integuments[b]	
Hair	201
Skin	28

[a] Data compiled from Hoekstra *et al.* (1956, 1967), Cassens *et al.* (1967), Miller *et al.* (1968), Crofton *et al.* (1983) and Zhou *et al.* (1994).

[b] Data of leucocytes, tissues and integuments are expressed as p.p.m. on a DM basis.

exchange ligands prior to analysis of Zn binding compounds (Jackson, 1989). Nevertheless, it has become apparent that Zn is part of many cellular metalloenzymes (Galdes & Vallee, 1983) and that Zn binds readily to the thiolate ligands present in the cellular protein metallothionein (Vasak & Kägi, 1983).

The distribution and nature of intracellular Zn is depicted in Fig. 1. Zinc is found in many cell compartments and is largely bound to cellular proteins (Williams, 1984). However, this does not exclude the existence of substantial amounts of free intracellular Zn or intracellular Zn complexed with one or more free amino acids (Jackson, 1989).

BIOLOGICAL FUNCTIONS OF INTRACELLULAR ZINC

To date, it has been suggested that Zn is involved in the following biological processes: (1) catalysis, (2) structural arrangement of protein, and (3) regulation of cellular events (Williams, 1989). For each of the processes, Zn exerts its biological activity almost entirely as part of complex molecules.

The catalytic function of Zn is clearly demonstrated in the enzyme carbonic anhydrase (Galdes & Vallee, 1983). The only physiological reaction known to be catalysed by carbonic anhydrase is the reversible hydration of carbon dioxide ($H_2O + CO_2 = H^+ + HCO_3^-$). The Zn ion of carbonic anhydrase is thought to participate in the first step of the catalytic reaction. Basically, Zn functions as an electron acceptor, or Lewis acid, and binds to the H_2O molecule. Due to the neutral imidazole ligands of the enzyme, the complex $Zn(H_2O)$ attains maximum acidity making ionization of H_2O to OH^- possible at pH 7 (Williams, 1989). The reactivity of the nucleophilic OH^- group is sufficient for carbonic anhydrase to attack the electrophilic CO_2 molecule. As a result, the end product HCO_3^- is formed (Galdes & Vallee, 1983). Zinc is thought to behave in a similar way in other Zn metalloenzymes which contain Zn at the active site.

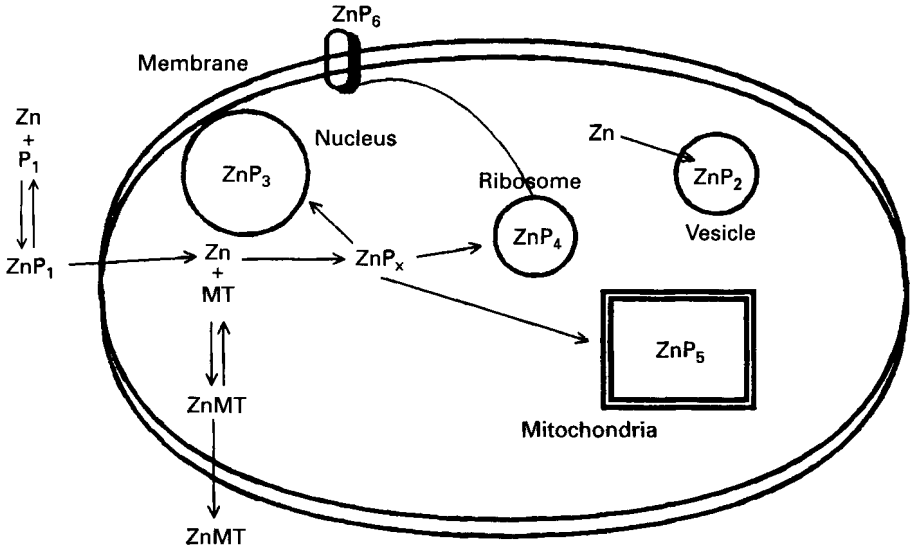

Fig. 1. Outline of the distribution of intracellular Zn (Williams, 1984). Zinc is carried to the cell by an extracellular protein carrier (ZnP_1). After being transported into the cell, free Zn can bind to intracellular metallothionein (MT) or other intracellular proteins (ZnP_x). Metallothionein binds Zn (ZnMT) and functions either as an intracellular storage protein or as an extracellular protein carrier. Zinc can also be transferred outside the cell as a constituent of enzymes or hormones (ZnP_2). Intracellularly, Zn is transported to different cellular compartments in which it carries out important biological functions. Nuclear (ZnP_3), ribosomal (ZnP_4) and mitochondrial (ZnP_5) proteins may be involved in polymerization, catalysis or protection. Zinc-containing enzymes or transporter proteins (ZnP_6), produced at the ribosomes, can be incorporated into the cell membrane.

Zinc may play a structural role in enzymes whenever it is located in a site not critical for catalysis. Furthermore, Zn is considered of critical importance in maintaining the structure of metalloproteins such as insulin and growth hormone. In this sense, Zn can be viewed as a replacement for a disulphide bond, a common feature in many proteins that provides stability by interlinking polypeptide chains (Stryer, 1988).

One disadvantage of the disulphide bond is that in a reducing environment the sulphur containing amino acids of the protein chain can be protonated. Protonation causes a breakup of the disulphide bridge so that protein conformation is lost. A second disadvantage of the disulphide bridge is that it allows little motion about itself and therefore restricts protein conformation. In contrast to the disulphide bridge, Zn cannot be reduced and puts very little stereochemical demand on the protein (Williams, 1984). Thus, Zn provides enzymes and other Zn-containing proteins with conformational stability at various pH without causing much steric hindrance.

A possible cooperative role of Zn and enzymes has been recognized in regulation of metabolic processes and synthesis (Jackson, 1989). Moreover, evidence has been presented that Zn is involved in gene expression of metallothionein (Seguin & Hamer, 1987). Recently, Cousins & Lee-Ambrose (1992) used rats to investigate the interactions of dietary Zn intake, nuclear Zn uptake and metallothionein gene expression. They reported that increases in dietary Zn were proportional to nuclear uptake of ingested Zn as well as to the level of metallothionein gene expression in the kidney, liver, intestine, spleen and heart. Using heparin-Sepharose chromatography and South-Western blotting, several Zn-binding protein fractions were isolated. One of the isolated protein fractions was able to bind an oligonucleotide in addition to Zn. This oligonucleotide was a DNA fragment of a

transcription factor of the metallothionein gene (Cousins & Lee-Ambrose, 1992). These results support the earlier findings of Seguin & Hamer (1987) that Zn is involved in regulation of metallothionein gene expression and suggest that this occurs in several tissues.

It is not always easy to distinguish among the catalytic, structural and regulatory functions of Zn. A good illustration is provided by Zn present in RNA and DNA polymerases. The nature of the function of Zn in these enzymes may be catalytic by binding substrate, primer or template. Alternatively, Zn may be involved in maintaining conformation and not be part of the active site of the enzymes. A third possibility is that Zn acts in a regulatory manner by supplying specificity to proteins involved in gene replication and transcription (Wu & Wu, 1983).

ZINC HOMEOSTASIS

Homeostasis can be considered effective when the animal is able to maintain optimum health and function (Aggett, 1991). Initially, the animal is able to maintain Zn homeostasis by varying the rates of Zn absorption and excretion (Fig. 2). In the short term the animal can further adjust its Zn status. At low dietary Zn intakes, redistribution of Zn occurs to those Zn pools that are important in metabolism. When dietary Zn intakes are high, Zn is sequestered in several body tissues such as liver and bone. Conditions of long term deprivation or excess of dietary Zn lead to inadequacy of processes involved in maintaining Zn homeostasis (Fig. 2).

The mechanisms which enable the animal to maintain Zn homeostasis are not exactly understood (Aggett, 1991). This lack of understanding is an important factor contributing to the problems encountered in determining Zn status.

ASSESSMENT OF ZINC STATUS

Levels of plasma or serum Zn and activities of Zn-containing metalloenzymes are frequently measured in studies with man (Prasad *et al.* 1971). When animals are used, these easily obtainable indicators of Zn status are often supplemented with measurements of Zn content and Zn metalloenzyme activities in various tissues (Giugliano & Millward, 1984). It is questionable, though, whether these indicators are sensitive enough to provide the accuracy required for a reliable interpretation of Zn status.

Plasma or serum contains only a small proportion of the whole body Zn content (Jackson, 1989). Moreover, Zn levels in plasma or serum respond directly to increases in dietary Zn intake and also to minor catabolic processes occurring in skeletal muscle and other tissues with large Zn stores.

There are clear effects of dietary Zn intake on tissue Zn levels, but they are not uniform among tissues. Feeding diets low in Zn leads to reductions in Zn concentrations of the pancreas, liver, kidney, heart, intestine, skin, hair and bones of the Zn depleted pigs compared with pigs pair-fed diets with National Research Council (1988) recommended levels of Zn (Hoekstra *et al.* 1956, 1967; Miller *et al.* 1968; Crofton *et al.* 1983; Dørup & Clausen, 1991). Zinc concentrations in skeletal muscle tissue, however, are not affected by dietary Zn levels (Crofton *et al.* 1983; Dørup & Clausen, 1991). The large Zn pool found in skeletal muscle appears to be important for the biological functioning of the animal.

The relationship between dietary Zn intake and tissue Zn levels was studied by Cousins & Lee-Ambrose (1992). Following an overnight fast, diets containing different levels of Zn were administered via a stomach tube to Zn adequate rats. Two hours after feeding, the largest portion of ^{65}Zn was found in the small intestinal tissue, followed by liver, bone

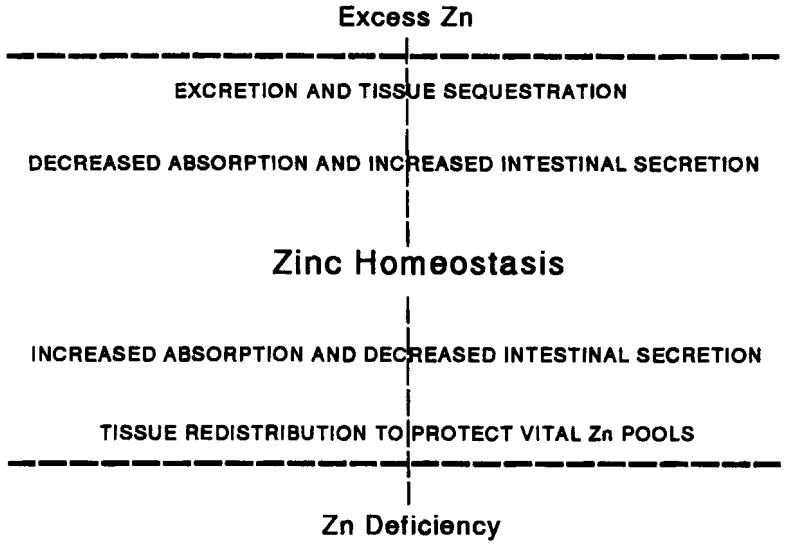

Fig. 2. Adaptive processes used to maintain Zn homeostasis (Aggett, 1991). During short periods of low or high dietary Zn intake, animals can initially maintain Zn homeostasis by adjusting intestinal Zn absorption and secretion. Other mechanisms that are enacted when intestinal mechanisms become insufficient are redistribution of Zn to tissues containing functional Zn pools and sequestration of Zn in tissues containing exchangeable Zn pools. During prolonged periods of low or high dietary Zn intake, animals cannot maintain Zn homeostasis and show signs of Zn deficiency and Zn toxicity respectively.

marrow, bone, skin, kidney, serum, thymus and skeletal muscle (Cousins & Lee-Ambrose, 1992).

It may be argued that pools which contain Zn needed for metabolic functions, the functional Zn pools, are located in those tissues and body fluids least affected by dietary Zn intake. Tissues that respond strongly to variations in dietary Zn intake may contain Zn pools less important for metabolism, the exchangeable Zn pools. The Zn content of many tissues and body fluids increases or decreases as dietary Zn intake changes. This suggests that there are numerous small sized exchangeable Zn pools.

In clinical nutrition, two approaches have been proposed to identify the early onset of Zn deficiency. The first involves prediction of exchangeable Zn pool sizes. This could be done by kinetic modelling studies in which either radioisotopes or the more widely used stable isotopes of Zn are used. Recently, Fairweather-Tait *et al.* (1993) demonstrated the feasibility of using an intravenous infusion of ^{70}Zn stable isotope and measuring plasma kinetics to estimate body pool sizes in man. They were able to distinguish between small rapidly exchanging and large slowly exchanging Zn pools containing less than 10 mg and approximately 350 mg of Zn respectively. Before this method can be used for assessment of Zn status, the relative sensitivity of body Zn pools to dietary and (or) physiological change must be established (Fairweather-Tait *et al.* 1993).

An alternative approach for identifying the exchangeable Zn pools is to diagnose factors involved in Zn redistribution between exchangeable and functional Zn pools. Some progress has been made in identifying a key factor involved in Zn redistribution, namely the Zn metalloprotein metallothionein (Golden, 1989). For accurately assessing Zn status in man, King (1990) proposed the use of a combination of plasma levels of Zn and metallothionein. Low plasma Zn and low metallothionein levels would indicate depletion of exchangeable Zn pools as a result of inadequate Zn intake. On the other hand, low plasma Zn in combination with high plasma metallothionein levels could be interpreted as

tissue redistribution of Zn from exchangeable to functional Zn pools. Although plasma Zn is low in both events, the latter condition is not necessarily caused by low dietary Zn intake (King, 1990). A balanced diet is not available to every human and most of the time there is a lack of information on dietary intake. Consequently, cases of Zn deficiency are frequently reported (Prasad, 1988), especially if Zn demands are increased due to growth or pregnancy (Yasodhara *et al.* 1991). The development of diagnostic tools which allow easy and accurate assessment of Zn status is essential. Without these indicators, it remains impossible to diagnose cases of Zn deficiency at an early stage.

In animal nutrition, optimum levels of Zn have been defined for the different domestic species. The US National Research Council (1979) and the UK Agricultural Research Council (1981) give recommended intakes, and diets are generally formulated accordingly. Therefore, Zn deficiency is rarely observed in modern livestock production and thus the assessment of Zn status is not common practice.

BIOLOGICAL ADAPTATIONS DURING ZINC DEFICIENCY

Experimentally depleted animals have been used to study the biological effects of Zn deficiency. Early investigations showed that Zn depletion reduces levels of Zn and Zn metalloenzymes in many tissues and body fluids (Hoekstra *et al.* 1956, 1967; Prasad *et al.* 1971). More recently, it was found that Zn deficiency causes increased osmotic fragility of red blood cell membranes (Johanning *et al.* 1990) and depression in both the humoral and cellular immune responses (Gupta *et al.* 1985; Verma *et al.* 1988; Spears *et al.* 1991).

Of particular interest are the studies investigating the relationships between dietary Zn intake and growth. Growth retardation observed at low intakes of Zn can only partly be accounted for by overall depression of feed intake. This was demonstrated by Miller *et al.* (1968). In their study, performance was determined for three groups of pigs receiving different amounts of dietary Zn. Pigs fed a Zn deficient diet had lower gains and poorer feed conversion efficiencies than both pair-fed controls and control pigs with *ad lib.* access to feed. Feed conversion efficiency was similar in the two control groups (Miller *et al.* 1968).

Growth retardation of Zn deficient animals has been associated with reductions in levels of blood insulin-like growth factor I (Cossack, 1986; Dørup *et al.* 1991), and insulin (Giugliano & Millward, 1987; Dørup *et al.* 1991; Droke *et al.* 1993). Moreover, decreases in serum mitogenic activity and depressions of total pituitary RNA levels and growth hormone mRNA expression were found in pigs fed low dietary levels of Zn (Swinkels *et al.* 1994*c*). The observed decreases in both pituitary growth hormone mRNA (Swinkels *et al.* 1994*c*) and blood insulin-like growth factor I levels (Cossack, 1986; Dørup *et al.* 1991; Droke *et al.* 1993) could not be linked to reduced serum growth hormone levels (Dørup *et al.* 1991). After injection of a growth hormone-releasing factor analogue, Droke *et al.* (1993) even observed an increase in serum growth hormone levels in Zn deficient lambs. Thus, more research is warranted to determine whether synthesis of growth hormone is affected by Zn deficiency and to identify those growth mechanisms in which Zn is of critical importance.

In fast growing animals, Zn deficiency primarily affects protein metabolism. Reduced protein accretion has been found to occur in skeletal muscle, heart, thymus (Giugliano & Millward, 1987; Dørup & Clausen, 1991), and small intestinal tissues (Southon *et al.* 1986) of Zn deficient animals compared with Zn adequate controls. A direct association between Zn metabolism and protein metabolism in tissues like the small intestine may be present.

The magnitude of the effects of Zn deficiency on metabolism is an indication of the importance of Zn as a nutrient. The changes in growth, gross anatomy and histology are directly related to inadequate supply of intracellular Zn. Consequently, levels and/or

activities of Zn metalloenzymes, Zn metalloproteins, and Zn transcription factors are reduced thereby impairing metabolism.

ABSORPTION OF ZINC

In general, absorption refers to one of the components in nutrient balance studies. More precisely, apparent absorption can be defined as the fraction of the dietary intake that does not appear in the faecal secretions. True absorption corrects the apparent absorption for endogenous losses occurring with intestinal secretions and mucosal sloughing which are not reabsorbed (O'Dell, 1984).

The process of Zn absorption can be physiologically divided into two separate events: firstly, uptake of Zn from the lumen into the cell, and secondly Zn transport from the cell into the circulatory system. In a review, Cousins (1989) has summarized current knowledge on mechanisms suspected to be involved in Zn uptake and transport (Fig. 3).

Uptake or cellular entry of Zn appears to occur by means of active transport and facilitated diffusion, both saturable processes (Davies, 1980; Menard & Cousins, 1983; Blakeborough & Salter, 1987). A small portion of Zn uptake and transport may be non-saturable, occurring through simple diffusion (Steel & Cousins, 1985) and paracellular movement of Zn, i.e. solvent drag (Bronner, 1987). The saturable uptake of Zn may involve binding of Zn by low molecular weight ligands which are present within the intestinal lumen. The Zn ligand complex either enters the cell intact or donates Zn to a membrane bound receptor. Subsequently, the receptor releases Zn intracellularly.

The capacity of the small intestine for Zn transport (V_{max}) depends on the body Zn status. Using isolated intestinal brush border membrane vesicles, Menard & Cousins (1983) found that Zn transport in rats fed adequate levels of Zn was twice as low as in Zn depleted rats. The affinity for Zn (K_m) was not affected by previous dietary Zn intakes. Thus, the increase in transport rate at low dietary Zn intakes is due only to an increase in number of receptors for free Zn or Zn bound to a low molecular weight ligand.

Evidence presented thus far suggests that Zn absorption is initiated largely by saturable uptake of Zn from the intestinal lumen into the cell. Using basolateral membrane vesicles of rat intestine, Oestreicher & Cousins (1989) studied transport of Zn out of the cell into the vascular system. Uptake of Zn in basolateral membrane vesicles was saturable and not affected by dietary Zn intake (Oestreicher & Cousins, 1989). A saturable Zn uptake indicates that a carrier mediated mechanism exists for Zn to enter the blood circulation. The lack of effect of dietary Zn intake on vesicular Zn uptake further suggests that Zn absorption is not regulated at the basolateral membrane (Oestreicher & Cousins, 1989).

ROLE OF INTESTINAL METALLOTHIONEIN IN ZINC ABSORPTION

Metallothionein is a protein of low molecular weight, about 6500 D, with a high metal-binding capacity, 7–10 atoms/mole (Bremner, 1983). For a thorough review of the role of metallothionein in Zn metabolism the reader is referred to Bremner (1983), Cousins (1985), Richards (1989) and Bremner & Beattie (1990).

A regulatory role of intestinal metallothionein in Zn absorption was first proposed by Richards & Cousins (1975). Intestinal metallothionein is synthesized in proportion to dietary Zn intake (Cousins & Lee-Ambrose, 1992). Metallothionein reduces Zn absorption by sequestering Zn within the enterocyte due to its higher affinity for Zn compared with other identified intestinal proteins (Starcher *et al.* 1980; Menard *et al.* 1981).

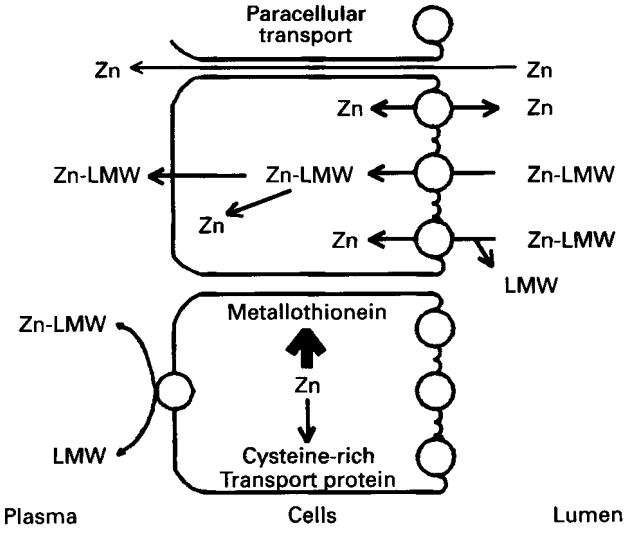

Fig. 3. A model for zinc absorption (Cousins, 1989; Hempe & Cousins, 1992). Zinc absorption can be physiologically divided into two processes: uptake of Zn from the GI lumen into the enterocyte (top) and transport of Zn from the enterocyte into the circulatory system (bottom). Within the GI lumen Zn may be presented for uptake into the enterocyte as free Zn or as Zn bound to a low molecular weight (LMW) ligand. The uptake of free Zn or Zn-LMW may involve carrier mediated and non-mediated mechanisms (top). Within the enterocyte, Zn transport may involve a cysteine-rich transcellular transport protein. Metallothionein competes for Zn with the transcellular transport protein and, therefore, may play a regulatory role in Zn absorption. Export of Zn from the enterocyte into the circulatory system may involve active mechanisms (bottom). A small portion of Zn uptake and transport may occur through simple diffusion and paracellular transport of free Zn (top).

As shown in Fig. 3, Hempe & Cousins (1992) included an interaction of a cysteine-rich intestinal protein, identified by Hempe & Cousins in 1989, with metallothionein in the Zn absorption model proposed by Cousins (1989). The cysteine-rich protein may enhance Zn absorption by transporting Zn transcellularly, from the intestinal brush border to the basolateral membrane. Intestinal metallothionein competitively inhibits binding of Zn to the transport protein and thereby regulates Zn absorption (Hempe & Cousins, 1992).

The regulatory role of intestinal metallothionein in Zn absorption has been partly challenged by Flanagan *et al.* (1983) and Coppen & Davies (1987). In these studies, criticism was focused on the proportional response of intestinal metallothionein synthesis to dietary Zn levels. Flanagan *et al.* (1983) observed only a transitory effect of dietary Zn level on intestinal metallothionein synthesis. They concluded that intestinal metallothionein synthesis was most likely induced by the nutritional stress associated with Zn deficiency and not by dietary Zn level (Flanagan *et al.* 1983). Moreover, Coppen & Davies (1987) found that dietary Zn intake did induce intestinal metallothionein synthesis in rats, but only at Zn levels of 5 to 80 mg per kg diet. At higher levels of Zn, 80 to 160 mg per kg diet, no further induction of intestinal metallothionein was observed (Coppen & Davies, 1987).

SITE(S) OF ZINC ABSORPTION

Many researchers have investigated the capacity for Zn absorption of various sites of the gut using a variety of methods. Some have reported that net Zn absorption in rats occurs primarily in the small intestine (Underwood, 1977) with negligible Zn absorption occurring

https://doi.org/10.1079/NRR19940009 Published online by Cambridge University Press

Table 2. *Apparent absorption coefficients of Zn determined within seven gut segments of depleted pigs fed an isolated soya protein semipurified Zn depletion diet supplemented with 15 and 45 p.p.m. Zn as $ZnSO_4$*[a]

gut segment[bc]	15 p.p.m. $ZnSO_4$	45 p.p.m. $ZnSO_4$	SEM
	%	%	
Stomach	−20·4	−16·1	6·6
Small intestine			
Proximal	−24·3	−46·9	18·7
Medial	5·4	0·7	14·1
Distal	25·5	17·6	5·8
Large intestine			
Caecum	18·0	18·4	4·7
Colon			
Proximal	20·3	15·7	4·9
Distal	16·7	17·4	6·2

[a] Apparent absorption coefficients were determined using the indirect indicator (0·25% Cr_2O_3) method. Each depleted mean represents five pigs that had been depleted for a 32-d period using an isolated soyabean semipurified diet containing 17 p.p.m. Zn. Pigs were killed 0, 3, 6, 12 and 24 d (one pig per d) after the start of a 24 d Zn repletion period. Digesta were collected exactly 2·5 h after feeding the last meal using a total digesta collection procedure. The gut was divided into seven segments: stomach, three small intestinal segments of equal length, caecum and two colonic segments of equal length.

[b] Linear, quadratic and cubic increases from stomach to distal colon segment ($P < 0·05$).

[c] Zn level by gut segment interaction ($P < 0·01$).

in other segments of the gut (Underwood, 1977; Davies, 1980). Others, however, did observe substantial Zn absorption in the large intestine of rats (Wapnir *et al.* 1985; Seal & Mathers, 1989), pigs (Partridge, 1978), sheep (Grace, 1975) and cattle (Bertoni *et al.* 1976). Furthermore, absorption of Zn anterior to the small intestine was observed in chickens (Miller & Jensen, 1966) and dairy cattle (Miller & Cragle, 1965).

Duodenal and ileal segments of the small intestine have been suggested as primary sites for Zn absorption. Infusion of ^{65}Zn into a ligated duodenal loop led to the highest ^{65}Zn recovery in blood, liver, kidneys and heart (Van Campen & Mitchell, 1965) or the whole body (Davies, 1980). With the use of an *in vivo* intestinal perfusion technique, it was found that the ileum had the highest capacity for Zn absorption (Antonson *et al.* 1979). Recently, Swinkels *et al.* (1994*b*) determined the site of apparent Zn absorption using a total digesta collection procedure and the indirect indicator method. The pigs used in this study had been depleted of Zn for a 5-week period by feeding an isolated soya protein semipurified diet. The diet contained 17 p.p.m. Zn and 3% cellulose. Following the depletion period, pigs were repleted by feeding the same diet supplemented with either 15 or 45 p.p.m. Zn as $ZnSO_4$. As shown in Table 2, jejunal and ileal segments of the small intestine were identified as the main sites of Zn absorption. The large intestine did not seem to contribute to the overall apparent Zn absorption (Swinkels *et al.* 1994*b*).

Partridge (1978) and Seal & Mathers (1989) examined the role of different amounts and sources of dietary fibre or non-starch polysaccharides on capacity and site of apparent Zn absorption. Using re-entrant cannulas in Zn adequate pigs given a casein semipurified diet containing about 50 p.p.m. Zn as $ZnCO_3$ and 3% cellulose, Partridge (1978) found that the large intestine was the primary site of Zn absorption. However, the gut sites anterior to the terminal ileum became the more important sites of apparent Zn absorption when 9% instead of 3% cellulose was included in the diet. The inclusion of a high level of cellulose in the diet also decreased overall apparent Zn absorption (Partridge, 1978). Feeding

different non-starch polysaccharide sources to rats, Seal & Mathers (1989) found similar rates of Zn absorption from everted gut sacs of duodenal, ileal and colonic segments. Analysis of the everted gut sacs showed that the absorbed Zn was largely accumulated in all intestinal tissues, particularly in the duodenal segment, and that only a small amount of Zn was transferred across the serosal surface. The source of non-starch polysaccharides in the diet appeared to affect the capacity of the large intestine for Zn absorption. Rats previously fed diets containing high levels of pectin showed higher rates of Zn transfer by colonic tissues than rats previously fed diets with or without non-starch polysaccharides from wheat bran (Seal & Mathers, 1989).

From the above mentioned studies it appears that all segments of the gut have the capacity to absorb Zn. As stated by Seal & Mathers (1989), some of the differences observed among the studies may relate to experimental technique, animal species and dietary composition. With regard to the diet, it appears that the capacity of the large intestine to absorb Zn is expressed when a fibre or non-starch polysaccharide source is included in the diet (Grace, 1975; Bertoni *et al.* 1976; Partridge, 1978; Seal & Mathers, 1989). However, inclusion of high amounts of dietary fibre or non-starch polysaccharide (Partridge, 1978) or a high body need for Zn in the experimental animals (Swinkels *et al.* 1994*b*) appeared to increase the relative contribution of the small intestine to overall Zn absorption.

MINERALS INTERACTING WITH ZINC DURING ABSORPTION

Transport of Zn from the intestinal lumen into the enterocyte can be impeded by other minerals. Iron and Cd have been shown to inhibit Zn uptake from an open-ended duodenal loop in Fe deficient mice (Hamilton *et al.* 1978). An interaction between Zn and Fe was also observed from jejunal segments of Fe adequate rats. Addition of Zn to the perfusate reduced absorption of Fe by 34% (El-Shobaki & Srour, 1989). Substantial inhibition exerted by Fe on Zn absorption in men was reported by Solomons & Jacob (1981). The inhibition became more apparent with increasing ratios of dietary Fe to Zn. In a subsequent study, it was shown that there is a competition between Fe and Zn at intraluminal and intracellular sites (Solomons *et al.* 1983).

Copper uptake by intestinal brush border membrane vesicles of rats given either high levels of Zn, adequate Cu and Zn, or Cu deficient diets was studied by Fischer & L'Abbe (1985). They observed the highest Cu uptake by vesicles of rats given high levels of Zn (Fischer & L'Abbe, 1985). In pigs given a high dietary level of Cu, an increase in plasma Cu and in liver Cu and Zn contents was observed together with a concurrent decrease in plasma and liver Fe (Shurson *et al.* 1990). A tissue specific association between Zn and Cu was found by Swinkels *et al.* (1994*a*). In their Zn depletion–repletion study, they examined the bioavailability of Zn from different Zn sources by determining the Zn contents in liver, kidney, pancreas, brain and gut tissues. In the kidney, Cu was depleted and replaced with Zn, whereas kidney Fe levels and both Cu and Fe levels in other tissues were not affected by the tissue Zn status (Swinkels *et al.* 1994*a*).

Zinc retention was not different in pigs fed different levels of Ca (Morgan *et al.* 1969). In rats, a negative effect of Zn on Ca uptake by intestinal brush border membrane vesicles was only observed at high Zn to Ca ratios (Roth-Bassell & Clydesdale, 1991).

It has become clear that the presence of Cu and Fe in the chyme can affect Zn absorption. It is not clear whether both minerals actually interfere with cellular uptake of Zn or whether Cu and Fe interact with Zn to form non-absorbable complexes within the lumen of the gut. Interference with cellular uptake may occur when the minerals compete either for common transporters in the cell membrane or for common cytosolic proteins that are involved in

intracellular transport of Zn. The interaction between Cu and Zn may result from competition for binding with metallothionein. Metallothionein has a high affinity for both Cu and Zn (Cousins, 1985), but thermodynamically binding with Cu is preferred to binding with Zn (Williams, 1984).

INTRINSIC FACTORS AFFECTING ZINC ABSORPTION

Zinc absorption is affected by dietary and endogenous factors. In rat jejunal segments, an excess of unhydrolysed glucose polymers and slowly absorbed sugars reduces Zn absorption (Wapnir *et al.* 1989). Absorption of Zn is also affected by the protein source (Miller & Jensen, 1966; O'Dell *et al.* 1972) as well as the protein concentration (Hunt & Larson, 1990; Hunt & Johnson, 1992).

The negative association of protein with Zn absorption may be due to other dietary components that contaminate the protein source. An allegedly higher Zn absorption from diets containing an animal protein source compared with diets based on cereal protein proved to be due to the presence of phytate in the cereal protein sources (Harmuth-Hoene & Meuser, 1987). Phytate and also fibre are two well known intrinsic factors which have been shown to affect the absorption of Zn negatively (Davies & Reid, 1979; Simons *et al.* 1990).

Endogenous factors may also interfere with Zn absorption from the gut lumen. A decrease in the plasma Zn level was observed by Sturniolo *et al.* (1991) after selective inhibition of gastric acid secretion in man. Their explanation was that a more alkaline environment in the stomach induces the formation of insoluble Zn compounds which cannot be absorbed further down the gut (Sturniolo *et al.* 1991).

As shown in the above mentioned studies both dietary and endogenous factors may affect the absorption of Zn to some extent but, unless dietary Zn intake is too low or too high for a prolonged period, the animal will be able to maintain Zn homeostasis.

BIOLOGICAL VALUE OF DIETARY COMPLEXES AND CHELATES OF ZINC

METAL COMPLEXES AND CHELATES

Metal complexes are compounds of a central metal atom together with ligands which contain at least one ligand atom with a free electron pair. Proteins and carbohydrates including their derivatives, lipids, and many synthetic compounds which contain an O, S or N atom may function as ligand (Kratzer & Vohra, 1986). The number of ligands that bind the metal atom usually exceeds the number expected from valency considerations (Smith, 1990). Binding of the ligand to the metal occurs through donation of the free electron pair of the ligand atom to the metal atom which acts as an electron acceptor (Fig. 4). This type of bond is referred to as a coordinate or dative bond. Coordinate bonds are mostly formed between the transitional elements and the electronegative atoms oxygen, sulphur and nitrogen (Kratzer & Vohra, 1986).

A metal chelate is a special form of a metal complex. A metal complex is considered a metal chelate when instead of one ligand atom, two or more atoms of the ligand donate their electron pairs to the metal in the formation of coordinate bonds. The chemical ring structure formed between the ligand and the metal resembles a pincer-like claw, for which the Greek word is 'Chēlē' (Fig. 4). Formation of metal complexes and chelates are both reversible processes. A continuous exchange of ligands occurs with a change in intraluminal or intracellular conditions. The free metal prefers those ligands with which it can form the

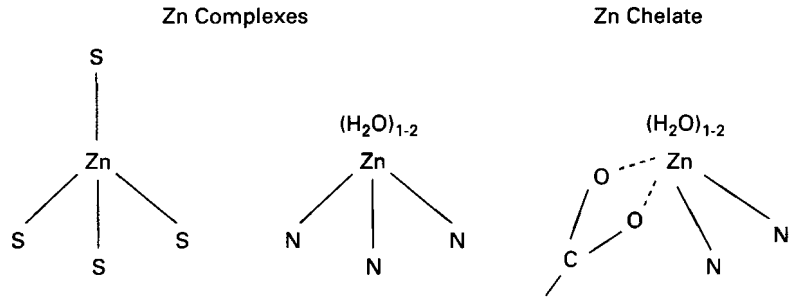

Fig. 4. Complexes and chelates of zinc as found at some known Zn sites of Zn-containing proteins (Williams, 1984). In Zn complexes only one atom of each participating ligand donates its free electron pair to Zn. this occurs at the structure sites of metallothionein (shown in part) and alcohol dehydrogenase (left figure) and at the active site of carbonic anhydrase (centre figure). In Zn chelates two or more atoms of each participating ligand donate their free electron pairs to Zn. This occurs at the active site of carboxypeptidases (right figure).

chemically most stable complexes or chelates under the given conditions (Kratzer & Vohra, 1986).

CONSIDERATIONS WHEN DETERMINING BIOAVAILABILITY OF ZINC

The term 'bioavailability' is generally used to describe the properties of absorption and utilization of nutrients (O'Dell, 1984). Only absorbed nutrients which can participate in the biological processes in the animal are considered utilizable, and thus bioavailable. Free Zn or Zn bound to low molecular weight ligands could have been absorbed and be present in body fluids and tissues, but it may not have been utilized. In determining the Zn availability of mineral sources, therefore, Zn metalloenzyme activities and metallothionein levels in body fluids and tissues are probably more reliable indicators of Zn availability than blood or tissue Zn concentrations.

A second important consideration in Zn availability studies is that animals maintain homeostasis by secreting part of the excess body Zn into the gut lumen. To prevent this mechanism from occurring, the animals should be depleted of Zn prior to the study. In a study conducted by Hallmans *et al.* (1987), bioavailability of Zn from one test food was estimated using two groups of rats with different needs for Zn to maintain Zn homeostasis. To increase the need for Zn in rats of the treatment group, anabolic processes that require Zn were stimulated by an intraperitoneal injection of a solution containing amino acids. The controls were injected with physiological saline. Results of the study showed that rats receiving the amino acid solution had a 40% increase in Zn absorption from the test food (Hallmans *et al.* 1987).

In addition to the use of animals with a high body need for Zn, the level of Zn used to replete the animals with Zn has to be carefully selected. If the levels of Zn are too low, the appetite of the depleted animals may remain depressed. On the other hand, if levels of Zn are too high, repletion of the depleted tissues with Zn may occur very rapidly. In both cases, the availability of Zn from the experimental diets cannot be determined. Thus, a dose response experiment has to be conducted to determine an optimum level of Zn. With an optimum Zn level, the change in Zn status of the depleted animal can be monitored over time as an indication of Zn availability in the experimental diets. Effectiveness and duration of Zn repletion can be determined by monitoring performance, repetitive measurement of serum or plasma Zn or metallothionein levels, the activities of metalloenzymes and serum

https://doi.org/10.1079/NRR19940009 Published online by Cambridge University Press

mitogenic activity (Miller *et al.* 1968; Prasad *et al.* 1971; Swinkels *et al.* 1994*a*). Moreover, tissue levels of Zn and Zn metalloenzyme activities can be determined at one or more times during Zn repletion. Each of these measurements or several of them together may be referred to as a Zn bioassay (Wedekind & Baker, 1990).

A final consideration in mineral availability studies is that the mineral source used as the control may influence the outcome of the comparison. For example, based on tibia Zn, Wedekind & Baker (1990) estimated a 61 % availability of Zn from ZnO relative to $ZnSO_4$. Both inorganic Zn sources are frequently used as control treatments in Zn bioassays.

AVAILABILITY OF ZINC COMPLEXED WITH PICOLINIC ACID

Picolinic acid (pyridine 2-carboxylic acid), a minor metabolite of tryptophan, was one of the first organic ligands studied for its possible promoting effect on Zn availability. The interest in picolinic acid originated from the finding that human milk provides more available Zn than cows' milk. In this research, Evans & Johnson (1979) characterized picolinic acid as a strong Zn binding ligand in human milk.

In subsequent studies, Evans & Johnson (1980*a–c*) showed that absorption of Zn was increased and growth rate stimulated in rats given diets supplemented with picolinic acid. In the later studies of Roth & Kirchgessner (1985) and Hill *et al.* (1986) these findings were not confirmed. Addition of picolinic acid to the diet improved neither the body gains, serum Zn levels, and Zn contents in the testes, femur and whole body of rats (Roth & Kirchgessner, 1985) nor bone Zn concentrations in pigs (Hill *et al.* 1986).

Using everted sacs of rat duodenum and ileum, Seal & Heaton (1983) studied the uptake of Zn with a variety of ligands including 2-picolinic and 4-picolinic acid. Salient features of this study are presented in Fig. 5. Adding 2-picolinic acid to the mixture improved the uptake of Zn in both everted duodenal and ileal sacs when compared with inorganic sulphate. Of the organic ligands tested, sulphate proved to be the most effective in enhancing Zn uptake. Compared to sulphate, the amino acids histidine and cysteine did improve Zn uptake from the ileal but not from the duodenal sac (Seal & Heaton, 1983). As part of the same study, the most promising ligands were included in the diets of intact rats housed in metabolism cages. The ligand 2-picolinic acid did improve apparent Zn absorption as expected from the results of the *in vitro* study. Zinc retention, however, was not improved primarily because of increased urinary Zn excretion (Seal & Heaton, 1983). These findings suggest that Zn was so tightly bound to 2-picolinic acid that it could not be utilized after being absorbed in complexed form, and therefore was excreted *via* the kidneys (Seal & Heaton, 1985).

AVAILABILITY OF ZINC COMPLEXED WITH METHIONINE OR OTHER AMINO ACIDS

Amino acids are frequently used as dietary ligands for synthesizing Zn complexes or chelates. Of all Zn amino acid complexes studied Zn methionine has received by far the most attention.

An improvement in Zn availability from the complex Zn methionine compared with $ZnSO_4$ was observed after measuring levels of Zn in the tibia of chicks (Wedekind *et al.* 1992). In other studies, Zn availability from Zn methionine, determined by measuring performance and serum Zn levels, was not different from an inorganic Zn salt in pigs (Kornegay & Thomas, 1975; Hill *et al.* 1986) or in heifers (Spears, 1989). Although apparent Zn absorption from Zn methionine was not different from ZnO, Spears (1989) did observe an increase in Zn retention of lambs fed Zn methionine. The increase in Zn

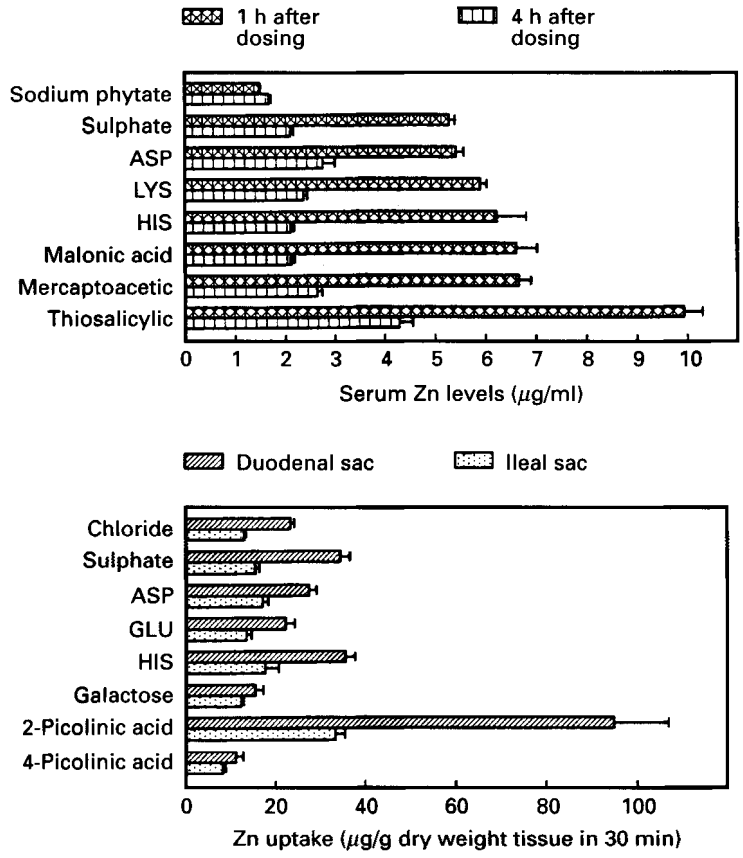

Fig. 5. Effect of inorganic and organic ligands on serum Zn concentrations and intestinal Zn uptake in rats. Serum Zn levels (top) were measured in rats 1 and 4 h after oral dosage of $ZnSO_4$ or mixtures of $ZnCl_2$ and different organic ligands containing 10 p.p.m. Zn (Giroux & Prakash, 1977). Intestinal Zn uptake (bottom) was measured using duodenal and ileal everted gut sacs after incubating with a buffer containing 0·0003 M-Zn as $ZnCl_2$, $ZnSO_4$ or mixtures of $ZnCl_2$ and organic ligands (Seal & Heaton, 1983). Organic ligands containing negatively charged carboxyl or thiol groups appeared to be the most effective in stimulating serum Zn concentrations and intestinal Zn uptake.

retention was caused by a slight reduction in urinary Zn excretion. A higher Zn retention with no difference in apparent Zn absorption (Spears, 1989) suggests that the complex Zn methionine provided more utilizable Zn than ZnO. In a 2-year study with beef cows and calves, Spears & Kegley (1991) observed a slight improvement in over-all performance with the use of Zn methionine and Mn methionine compared with ZnO and MnO. Addition of Zn methionine instead of ZnO to chick diets containing already adequate amounts of Zn slightly increased the content of Zn in the pancreas (Pimentel *et al.* 1991). Growth and concentrations of Zn in tibiotarsus and liver were not affected by the Zn source (Pimentel *et al.* 1991). Carcass quality of steers was improved with a Zn methionine supplement to the diet instead of ZnO (Greene *et al.* 1988). The better carcass quality was not associated with improved performance.

The influence of a variety of amino acids and their chemical homologues on Zn uptake from perfused jejunal, ileal and colonic segments of rats was studied by Wapnir & Stiel (1986). In the small intestine, perfusion with tryptophan, histidine, cysteine and proline achieved a higher Zn uptake compared with results after perfusion with their respective

https://doi.org/10.1079/NRR19940009 Published online by Cambridge University Press

homologues tryptophol, imidazole, *N*-acetyl-L-cysteine and pyroglutamate. It appeared that both mediated and non-mediated transport mechanisms were involved in Zn uptake of the small intestine when the perfusate contained one of the amino acids. When the perfusate contained one of the amino acid homologues only non-mediated transport mechanisms appeared to be activated. In the colon, uptake of Zn was increased only with imidazole, the homologue of histidine, which may be explained by the high structural affinity of imidazole for Zn (Wapnir & Stiel, 1986). In humans, a 25% increase in serum Zn level was observed after ingestion of Zn as a Zn histidine complex compared with $ZnSO_4$ (Schölmerich *et al.* 1987). The apparent higher absorption of Zn histidine, however, was associated with increased urinary Zn excretion. Performance of grower pigs was not improved by supplementing Zn adequate diets with 1% histidine or 289 p.p.m. EDTA (Dahmer *et al.* 1972; Owen *et al.* 1973). However, inclusion of histidine appeared to alleviate skin lesions of the Zn deficient pigs used by Dahmer *et al.* (1972). This may indicate that dietary histidine did improve Zn availability. Alternatively, histidine may have stimulated the healing process independent of Zn. After measuring several serum and tissue variables in pigs that had been depleted of Zn, Swinkels *et al.* (1994*a*) reported similar availabilities of Zn from an amino acid chelate and $ZnSO_4$. However, the apparent Zn absorption coefficients were not consistent with the serum and tissue Zn measurements (Swinkels *et al.* 1994*b*).

Uptake, mucosal retention and absorption of Zn from $ZnCl_2$, $ZnCl_2$ with methionine, Zn complexes with methionine and $ZnCl_2$ with EDTA were studied by Hempe & Cousins (1989) with the aid of ligated rat duodenal loops. After 60 min incubation, Zn uptake and absorption were lowest for Zn methionine and the Zn EDTA mixture. It was suggested that the low Zn absorption was associated with reduced binding of Zn to an unidentified low molecular weight protein present in the mucosa (Hempe & Cousins, 1989). Later on, the protein was assigned a possible role in the transcellular transport of Zn as shown in Fig. 3. Uptake of Zn, determined with everted duodenal sacs of pigs, was not different among $ZnSO_4$, Zn methionine and Zn lysine (Hill *et al.* 1987).

The hypothesis that organic ligands are actively involved in Zn absorption was examined by Giroux & Prakash (1977). Salient features of the results of this study are shown in Fig. 5. In their study, Giroux & Prakash (1977) gave different ligand and $ZnSO_4$ mixtures (10 p.p.m. Zn) by stomach tube after a 24 h fast. One and 4 h after force feeding the rats, they determined Zn absorption by measuring serum Zn levels. As shown in Fig. 5, ligands containing thiol and carboxylic acid groups, such that formation of five- or six-membered Zn chelates could occur, proved to be the most effective in increasing levels of serum Zn 1 h after feeding. A 1:1 mixture of phytate and $ZnSO_4$ reduced serum Zn levels about 3·5 times compared with the control $ZnSO_4$. Of the amino acids tested, a 2:1 mixture of glycine and $ZnSO_4$ was most effective. Increases in serum Zn levels observed with the amino acids lysine, histidine and cysteine were only slightly lower. Serum Zn levels, measured 4 h after feeding, returned to the $ZnSO_4$ control value for most amino acid and $ZnSO_4$ mixtures. In contrast to the amino acid ligands, changes in levels of serum Zn observed with phytate and several ligands containing thiol or carboxylic acid groups 1 h after feeding were still maintained 4 h after feeding (Giroux & Prakash, 1977). Also, in the study of Seal & Heaton (1983), a ligand containing a carboxylic acid group, 2-picolinic acid, improved the uptake of Zn when compared with inorganic sulphate (Fig. 5).

It is difficult to deduce a general mode of action for absorption of organic Zn complexes or chelates from the studies of Giroux & Prakash (1977) and Seal & Heaton (1983). In both studies it appears, however, that absorption of Zn may be enhanced in the presence of organic ligands containing highly negatively charged atom groups that form negatively charged Zn complexes or chelates. This observation is supported by the findings of Tacnet

et al. (1990) in a study using intestinal membrane vesicles of rats. They also found a considerable increase in vesicular Zn uptake when $ZnSO_4$ was substituted for $Zn(SCN)_4^{2-}$, a highly negatively charged anion.

CONCLUSIONS

Studies on the mechanisms underlying Zn absorption have suggested that Zn may be absorbed as part of an intact complex or chelate formed between Zn and one or more organic ligands. To date, this hypothesis has been examined primarily using Zn complexed to either picolinate or methionine. Although both organic Zn forms have been shown to affect Zn absorption, a consistent improvement in availability of Zn from these sources was not found. More basic research focusing on conditions within the gut lumen and on mechanisms underlying Zn absorption is needed to elucidate which characteristics of a dietary Zn form are essential in determining its biological value. Within the gut lumen, dietary Zn complexes or chelates should be stable enough to withstand luminal conditions in all gut segments prior to the site of absorption. In order to be utilized, the stability of the dietary organic Zn form should be low enough to allow for the release or donation of Zn either during or after Zn absorption. Previous research has shown that dietary ligands may affect both absorption and utilization of Zn. A complete understanding of the mechanisms underlying these processes, however, is necessary to determine the specific importance of ligands. When these mechanisms are elucidated, it should be possible to develop dietary Zn complexes or chelates with a high biological value more effectively.

REFERENCES

Aggett, P. J. (1991). The assessment of zinc status: a personal view. *Proceedings of the Nutrition Society* **50**, 9–17.

Agricultural Research Council. (1981). *The Nutrient Requirements of Pigs*. Farnham Royal: Commonwealth Agricultural Bureaux.

Antonson, D. L., Barak, A. J. & Vanderhoof, J. A. (1979). Determination of the site of zinc absorption in rat small intestine. *Journal of Nutrition* **109**, 142–147.

Bartholomew, M. E., Tupper, R. & Wormall, A. (1959). Incorporation of ^{65}Zn in the sub-cellular fractions of the liver and spontaneously occurring mammary tumours of mice after the injection of zinc-glycine containing ^{65}Zn. *Biochemical Journal* **73**, 256–261.

Bertoni, G., Watson, M. J., Savage, G. P. & Armstrong, D. G. (1976). [The movements of minerals in the digestive tract of dry and lactating Jersey cows. 2. Net movements of Cu, Fe, Mn and Zn.] *Zootecnica e Nutrizione Animale* **2**, 185–191.

Blakeborough, P. & Salter, D. N. (1987). The intestinal transport of zinc studied using brush-border-membrane vesicles from the piglet. *British Journal of Nutrition* **57**, 45–55.

Bremner, I. (1983). The roles of metallothionein in the metabolism of copper and zinc. *Annual Report of Studies in Animal Nutrition and Allied Sciences, Rowett Research Institute* **39**, 13–28.

Bremner, I. & Beattie, J. H. (1990). Metallothionein and the trace minerals. *Annual Review of Nutrition* **10**, 63–83.

Bronner, F. (1987). Intestinal calcium absorption: mechanisms and applications. *Journal of Nutrition* **117**, 1347–1352.

Cassens, R. G., Hoekstra, W. G., Faltin, E. C. & Briskey, E. J. (1967). Zinc content and subcellular distribution in red vs. white porcine skeletal muscle. *American Journal of Physiology* **212**, 688–692.

Coppen, D. E. & Davies, N. T. (1987). Studies on the effects of dietary zinc dose on ^{65}Zn absorption in vivo and on the effects of Zn status on ^{65}Zn absorption and body loss in young rats. *British Journal of Nutrition* **57**, 35–44.

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.

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. (1989). Theoretical and practical aspects of zinc uptake and absorption. In *Mineral Absorption in the Monogastric GI Tract*, pp. 3–12 [F. R. Dintzis and J. A. Laszlo, editors]. New York, NY: Plenum Press.

Cousins, R. J. & Lee-Ambrose, L. M. (1992). Nuclear zinc uptake and interactions and metallothionein gene expression are influenced by dietary zinc in rats. *Journal of Nutrition* **122**, 56–64.

Crofton, R. W., Clapham, M., Humphries, W. R., Aggett, P. J. & Mills, C. F. (1983). Leucocyte and tissue zinc concentrations in the growing pig. *Proceedings of the Nutrition Society* **42**, 128A (Abstr.).

Dahmer, E. J., Coleman, B. W., Grummer, R. H. & Hoekstra, W. G. (1972). Alleviation of parakeratosis in zinc deficient swine by high levels of dietary histidine. *Journal of Animal Science* **35**, 1181–1189.
Davies, N. T. (1980). Studies on the absorption of zinc by rat intestine. *British Journal of Nutrition* **43**, 189–203.
Davies, N. T. & Reid, H. (1979). An evaluation of the phytate, zinc, copper, iron and manganese contents of, and Zn availability from, soya-based textured-vegetable-protein meat-substitutes or meat-extenders. *British Journal of Nutrition* **41**, 579–589.
Dørup, I. & Clausen, T. (1991). Effects of magnesium and zinc deficiencies on growth and protein synthesis in skeletal muscle and the heart. *British Journal of Nutrition* **66**, 493–504.
Dørup, I., Flyvbjerg, A., Everts, M. E. & Clausen, T. (1991). Role of insulin-like growth factor-1 and growth hormone in growth inhibition induced by magnesium and zinc deficiencies. *British Journal of Nutrition* **66**, 505–521.
Droke, E. A., Spears, J. W., Armstrong, J. D., Kegley, E. B. & Simpson, R. B. (1993). Dietary zinc affects serum concentrations of insulin and insulin-like growth factor I in growing lambs. *Journal of Nutrition* **123**, 13–19.
El-Shobaki, F. A. & Srour, M. G. (1989). The influence of ascorbic acid and lactose on the interaction of iron with each of cobalt and zinc during intestinal absorption. *Zeitschrift fur Ernährungswissenschaft* **28**, 310–315.
Evans, G. W. & Johnson, E. C. (1980*a*). Zinc absorption in rats fed a low-protein diet and a low-protein diet supplemented with tryptophan or picolinic acid. *Journal of Nutrition* **110**, 1076–1080.
Evans, G. W. & Johnson, E. C. (1980*b*). Zinc concentration of liver and kidneys from rat pups nursing dams fed supplemental zinc dipicolinate or zinc acetate. *Journal of Nutrition* **110**, 2121–2124.
Evans, G. W. & Johnson, E. C. (1980*c*). Growth stimulating effect of picolinic acid added to rat diets. *Proceedings of the Society for Experimental Biology and Medicine* **165**, 457–461.
Evans, G. W. & Johnson, P. E. (1979). Purification and characterization of a zinc-binding ligand in human milk. *Federation Proceedings* **38**, 703.
Fairweather-Tait, S. J., Jackson, M. J., Fox, T. E., Wharf, S. G., Eagles, J. & Croghan, P. C. (1993). The measurement of exchangeable pools of zinc using the stable isotope ^{70}Zn. *British Journal of Nutrition* **70**, 221–234.
Fischer, P. W. F. & L'Abbe, M. R. (1985). Copper transport by intestinal brush border membrane vesicles from rats fed high zinc or copper deficient diets. *Nutrition Research* **5**, 759–767.
Flanagan, P. R., Haist, J. & Valberg, L. S. (1983). Zinc absorption, intraluminal zinc and intestinal metallothionein levels in zinc-deficient and zinc-repleted rodents. *Journal of Nutrition* **113**, 962–972.
Galdes, A. & Vallee, B. L. (1983). Categories of zinc metalloenzymes. In *Zinc and its Role in Biology and Nutrition* (*Metal Ions in Biological Systems* vol. 15), pp. 1–54 [H. Sigel and A. Sigel, editors]. New York: Marcel Dekker, Inc.
Giroux, E. & Prakash, N. J. (1977). Influence of zinc-ligand mixtures on serum zinc levels in rats. *Journal of Pharmaceutical Sciences* **66**, 391–395.
Giugliano, R. & Millward, D. J. (1984). Growth and zinc homeostasis in the severely Zn-deficient rat. *British Journal of Nutrition* **52**, 545–560.
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.
Golden, B. E. (1989). Zinc in cell division and tissue growth: physiological aspects. In *Zinc in Human Biology*, pp. 119–128 [C. F. Mills, editor]. London: Springer-Verlag.
Grace, N. D. (1975). Studies on the flow of zinc, cobalt, copper and manganese along the digestive tract of sheep given fresh perennial ryegrass, or white or red clover. *British Journal of Nutrition* **34**, 73–82.
Grace, N. D. (1983). Amounts and distribution of mineral elements associated with fleece-free empty body weight gains in the grazing sheep. *New Zealand Journal of Agricultural Research* **26**, 59–70.
Greene, L. W., Lunt, D. K., Byers, F. M., Chirase, N. K., Richmond, C. E., Knutson, R. E. & Schelling, G. T. (1988). Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. *Journal of Animal Science* **66**, 1818–1823.
Gupta, R. P., Verma, P. C. & Gupta, R. K. P. (1985). Experimental zinc deficiency in guinea-pigs: clinical signs and some haematological studies. *British Journal of Nutrition* **54**, 421–428.
Hallmans, G., Nilsson, U., Sjöstrom, R., Wetter, L. & Wing, K. (1987). The importance of the body's need for zinc in determining Zn availability in food: a principle demonstrated in the rat. *British Journal of Nutrition* **58**, 59–64.
Hambidge, K. M., Casey, C. E. & Krebs, N. F. (1986). Zinc. In *Trace Elements in Human and Animal Nutrition*, 5th Edn, vol. 2, pp. 1–137 [W. Mertz, editor]. London: Academic Press.
Hamilton, D. L., Bellamy, J. E. C., Valberg, J. D. & Valberg, L. S. (1978). Zinc, cadmium, and iron interactions during intestinal absorption in iron-deficient mice. *Canadian Journal of Physiology and Pharmacology* **56**, 384–389.
Harmuth-Hoene, A. E. & Meuser, F. (1987). [Biological availability of zinc in whole-grain cereal products with various phytate contents.] *Zeitschrift für Ernährungswissenschaft* **26**, 250–267.
Hempe, J. M. & Cousins, R. J. (1989). Effect of EDTA and zinc-methionine complex on zinc absorption by rat intestine. *Journal of Nutrition* **119**, 1179–1187.
Hempe, J. M. & Cousins, R. J. (1992). Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. *Journal of Nutrition* **122**, 89–95.

Hill, D. A., Peo, E. R. & Lewis, A. J. (1987). Influence of picolinic acid on the uptake of ^{65}Zn-amino acid complexes by the everted rat gut. *Journal of Animal Science* **65**, 173–178.

Hill, D. A., Peo, E. R., Lewis, A. J. & Crenshaw, J. D. (1986). Zinc-amino acid complexes for swine. *Journal of Animal Science* **63**, 121–130.

Hoekstra, W. G., Faltin, E. C., Lin, C. W., Roberts, H. F. & Grummer, R. H. (1967). Zinc deficiency in reproducing gilts fed a diet high in calcium and its effect on tissue zinc and blood serum alkaline phosphatase. *Journal of Animal Science* **26**, 1348–1357.

Hoekstra, W. G., Lewis, P. K., Phillips, P. H. & Grummer, R. H. (1956). The relationship of parakeratosis, supplemental calcium and zinc to the zinc content of certain body components of swine. *Journal of Animal Science* **15**, 752–764.

Hunt, J. R. & Johnson, L. K. (1992). Dietary protein, as egg albumen: effects on bone composition, zinc bioavailability and zinc requirements of rats, assessed by a modified broken-line model. *Journal of Nutrition* **122**, 161–169.

Hunt, J. R. & Larson, B. J. (1990). Meal protein and zinc levels interact to influence zinc retention by the rat. *Nutrition Research* **10**, 697–705.

Jackson, M. J. (1989). Physiology of zinc: general aspects. In *Zinc in Human Biology*, pp. 1–14 [C. F. Mills, editor]. London: Springer-Verlag.

Johanning, G. L., Browning, J. D., Bobilya, D. J., Veum, T. L. & O'Dell, B. L. (1990). Effect of zinc deficiency and food restriction in the pig on erythrocyte fragility and plasma membrane composition. *Nutrition Research* **10**, 1463–1471.

Kernkamp, H. C. H. & Ferrin, E. F. (1953). Parakeratosis in swine. *Journal of American Veterinary Medical Association* **123**, 217–220.

King, J. C. (1990). Assessment of zinc status. *Journal of Nutrition* **120**, 1474–1479.

Kornegay, E. T. & Thomas, H. R. (1975). Zinc-proteinate supplement studied. *Hog Farm Management* (August), 50–51.

Kratzer, F. H. & Vohra, P. (1986). *Chelates in Nutrition*. Boca Raton, FL: CRC Press, Inc.

Menard, M. P. & Cousins, R. J. (1983). Zinc transport by brush border membrane vesicles from rat intestine. *Journal of Nutrition* **113**, 1434–1442.

Menard, M. P., McCormick, C. C. & Cousins, R. J. (1981). Regulation of intestinal metallothionein biosynthesis in rats by dietary zinc. *Journal of Nutrition* **111**, 1353–1361.

Miller, E. R., Luecke, R. W., Ullrey, D. E., Baltzer, B. V., Bradley, B. L. & Hoefer, J. A. (1968). Biochemical, skeletal and allometric changes due to zinc deficiency in the baby pig. *Journal of Nutrition* **95**, 278–286.

Miller, J. K. & Cragle, R. G. (1965). Gastrointestinal sites of absorption and endogenous secretion of zinc in dairy cattle. *Journal of Dairy Science* **48**, 370–373.

Miller, J. K. & Jensen, L. S. (1966). Effect of dietary protein source on zinc absorption and excretion along the alimentary tracts of chicks. *Poultry Science* **45**, 1051–1053.

Miller, W. J., Neathery, M. W., Gentry, R. P., Blackmon, D. M. & Stake, P. E. (1974). Adaptations in zinc metabolism by lactating cows fed a low-zinc practical-type diet. In *Trace Element Metabolism in Animals-2*, pp. 550–552 [W. G. Hoekstra, J. W. Suttie, H. E. Ganther and W. Mertz, editors]. Baltimore, MD: University Park Press.

Morgan, D. P., Young, E. P., Earle, I. P., Davey, R. J. & Stevenson, J. W. (1969). Effects of dietary calcium and zinc on calcium, phosphorus and zinc retention in swine. *Journal of Animal Science* **29**, 900–905.

National Research Council, Subcommittee on Zinc. (1979). *Zinc*. Baltimore, MD: University Park Press.

National Research Council. (1988). *Nutrient Requirements of Swine*, 10th ed. Washington, DC: National Academy Press.

O'Dell, B. L. (1984). Bioavailability of trace elements. *Nutrition Reviews* **42**, 301–308.

O'Dell, B. L., Burpo, C. E. & Savage, J. E. (1972). Evaluation of zinc availability in foodstuffs of plant and animal origin. *Journal of Nutrition* **102**, 653–660.

Oestreicher, P. & Cousins, R. J. (1989). Zinc uptake by basolateral membrane vesicles from rat small intestine. *Journal of Nutrition* **119**, 639–646.

Owen, A. A., Peo, E. R., Cunningham, P. J. & Moser, B. D. (1973). Effect of EDTA on utilization of dietary zinc by G-F swine. *Journal of Animal Science* **37**, 470–478.

Pallauf, J., Höhler, D. & Rimbach, G. (1992). [Effect of microbial phytase supplementation to a maize-soya diet on the apparent absorption of Mg, Fe, Cu, Mn and Zn and parameters of Zn status in piglets]. *Journal of Animal Physiology and Nutrition* **68**, 1–9.

Partridge, I. G. (1978). Studies on digestion and absorption in the intestines of growing pigs. 4. Effects of dietary cellulose and sodium levels on mineral absorption. *British Journal of Nutrition* **39**, 539–545.

Pimentel, J. L., Cook, M. E. & Greger, J. L. (1991). Bioavailability of zinc-methionine for chicks. *Poultry Science* **70**, 1637–1639.

Prasad, A. S. (1988). Zinc in growth and development and spectrum of human zinc deficiency. *Journal of the American College of Nutrition* **7**, 377–384.

Prasad, A. S., Oberleas, D., Miller, E. R. & Luecke, R. W. (1971). Biochemical effects of zinc deficiency: changes in activities of zinc-dependent enzymes and ribonucleic acid and deoxyribonucleic acid content of tissues. *Journal of Laboratory and Clinical Medicine* **77**, 144–152.

Richards, M. P. (1989). Recent developments in trace element metabolism and function: role of metallothionein in copper and zinc metabolism. *Journal of Nutrition* **119**, 1062–1070.
Richards, M. P. & Cousins, R. J. (1975). Mammalian zinc homeostasis: requirement for RNA and metallothionein synthesis. *Biochemical and Biophysical Research Communications* **64**, 1215–1223.
Roth, H. P. & Kirchgessner, M. (1985). Utilization of zinc from picolinic or citric acid complexes in relation to dietary protein source in rats. *Journal of Nutrition* **115**, 1641–1649.
Roth-Bassell, H. A. & Clydesdale, F. M. (1991). The influence of zinc, magnesium, and iron on calcium uptake in brush border membrane vesicles. *Journal of the American College of Nutrition* **10**, 44–49.
Schölmerich, J., Freudemann, A., Köttgen, E., Wietholz, H., Steiert, B., Löhle, B., Häussinger, D. & Gerok, W. (1987). Bioavailability of zinc from zinc-histidine complexes. I. Comparison with zinc sulfate in healthy men. *American Journal of Clinical Nutrition* **45**, 1480–1486.
Seal, C. J. & Heaton, F. W. (1983). Chemical factors affecting the intestinal absorption of zinc in vitro and in vivo. *British Journal of Nutrition* **50**, 317–324.
Seal, C. J. & Heaton, F. W. (1985). Effect of dietary picolinic acid on the metabolism of exogenous and endogenous zinc in the rat. *Journal of Nutrition* **115**, 986–993.
Seal, C. J. & Mathers, J. C. (1989). Intestinal zinc transfer by everted gut sacs from rats given diets containing different amounts and types of dietary fibre. *British Journal of Nutrition* **62**, 151–163.
Seguin, C. & Hamer, D. H. (1987). Regulation in vitro of metallothionein gene binding factors. *Science* **235**, 1383–1387.
Shurson, G. C., Ku, P. K., Waxler, G. L., Yokoyama, M. T. & Miller, E. R. (1990). Physiological relationships between microbiological status and dietary copper levels in the pig. *Journal of Animal Science* **68**, 1061–1071.
Simons, P. C. M., Versteegh, H. A. J., Jongbloed, A. W., Kemme, P. A., Slump, P., Bos, K. D., Wolters, M. G. E., Beudeker, R. F. & Verschoor, G. J. (1990). Improvement of phosphorus availability by microbial phytase in broilers and pigs. *British Journal of Nutrition* **64**, 525–540.
Smith, D. W. (1990). *Inorganic Substances*. Cambridge: University Press.
Solomons, N. W. & Jacob, R. A. (1981). Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc. *American Journal of Clinical Nutrition* **34**, 475–482.
Solomons, N. W., Pineda, O., Viteri, F. & Sandstead, H. H. (1983). Studies on the bioavailability of zinc in humans: mechanism of the intestinal interaction of nonheme iron and zinc. *Journal of Nutrition* **113**, 337–349.
Southon, S., Gee, J. M., Bayliss, C. E., Wyatt, G. M., Horn, N. & Johnson, I. T. (1986). Intestinal microflora, morphology and enzyme activity in zinc-deficient and Zn-supplemented rats. *British Journal of Nutrition* **55**, 603–611.
Spears, J. W. (1989). Zinc methionine for ruminants: relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. *Journal of Animal Science* **67**, 835–843.
Spears, J. W. & Kegley, E. B. (1991). Effect of zinc and manganese methionine on performance of beef cows and calves. *Journal of Animal Science* **69** Suppl. 1, 59.
Spears, J. W., Kegley, E. B. & Ward, J. D. (1991). Bioavailability of organic, inorganic trace minerals explored. *Feedstuffs* (November), 12–20.
Spray, C. M. & Widdowson, E. M. (1950). The effect of growth and development on the composition of mammals. *British Journal of Nutrition* **4**, 332–352.
Starcher, B. C., Glauber, J. G. & Madaras, J. G. (1980). Zinc absorption and its relationship to intestinal metallothionein. *Journal of Nutrition* **110**, 1391–1397.
Steel, L. & Cousins, R. J. (1985). Kinetics of zinc absorption by luminally and vascularly perfused rat intestine. *American Journal of Physiology* **248**, G46–G53.
Stryer, L. (1988). *Biochemistry*, 3rd ed. New York, NY: W. H. Freeman and Co.
Sturniolo, G. C., Montino, C., Rossetto, L., Martin, A., D'Inca, R., D'Odorico, A. & Naccarato, R. (1991). Inhibition of gastric acid secretion reduces zinc absorption in man. *Journal of American College of Nutrition* **10**, 372–375.
Swinkels, J. W. G. M., Kornegay, E. T., Zhou, W., Lindemann, M. D., Webb, K. E. & Verstegen, M. W. A. (1994*a*). Effectiveness of a zinc amino acid chelate and zinc sulfate in repleting serum and soft tissue zinc pools when fed to zinc depleted pigs. *Journal of Animal Science*, submitted.
Swinkels, J. W. G. M., Kornegay, E. T., Zhou, W., Lindemann, M. D., Webb, K. E. & Verstegen, M. W. A. (1994*b*). In vivo assessment of rate and site of apparent zinc, copper and iron absorption as affected by Zn source using Zn depleted pigs. *Journal of Animal Science*, submitted..
Swinkels, J. W. G. M., Kornegay, E. T., Zhou, W., Wong, E. A., Lindemann, M. D. & Verstegen, M. W. A. (1994*c*). Serum mitogenic activity, total pituitary RNA and growth hormone mRNA concentrations of experimentally zinc depleted pigs. *Journal of Nutrition* (In press).
Tacnet, F., Watkins, D. W. & Ripoche, P. (1990). Studies of zinc transport into brush-border membrane vesicles isolated from pig small intestine. *Biochimica et Biophysica Acta* **1024**, 323–330.
Todd, W. R., Elvehjem, C. A. & Hart, E. B. (1934). Zinc in the nutrition of the rat. *American Journal of Physiology* **107**, 146–156.
Tucker, H. F. & Salmon, W. D. (1955). Parakeratosis or zinc deficiency disease in the pig. *Proceedings of the Society for Experimental Biology and Medicine* **88**, 613–616.
Underwood, E. J. (1977). *Trace Elements in Human and Animal Nutrition*, 4th ed. London: Academic Press.

Van Campen, D. R. & Mitchell, E. A. (1965). Absorption of Cu^{64}, Zn^{65}, Mo^{99}, and Fe^{59} from ligated segments of the rat gastrointestinal tract. *Journal of Nutrition* **86**, 120–124.

Vasak, M. & Kägi, J. H. R. (1983). Spectroscopic properties of metallothionein. In *Zinc and its Role in Biology and Nutrition* (*Metal Ions in Biological Systems* vol. 15), pp. 213–273 [H. Sigel and A. Sigel, editors]. New York, NY: Marcel Dekker, Inc.

Verma, P. C., Gupta, R. P., Sadana, J. R. & Gupta, R. K. P. (1988). Effect of experimental zinc deficiency and repletion on some immunological variables in guinea-pigs. *British Journal of Nutrition* **59**, 149–154.

Wapnir, R. A., Garcia-Aranda, J. A., Mevorach, D. E. K. & Lifshitz, F. (1985). Differential absorption of zinc and low-molecular-weight ligands in the rat gut in protein-energy malnutrition. *Journal of Nutrition* **115**, 900–908.

Wapnir, R. A. & Stiel, L. (1986). Zinc intestinal absorption in rats: specificity of amino acids as ligands. *Journal of Nutrition* **116**, 2171–2179.

Wapnir, R. A., Stiel, L. & Lee, S.-Y. (1989). Zinc intestinal absorption: effect of carbohydrates. *Nutrition Research* **9**, 1277–1284.

Wedekind, K. J. & Baker, D. H. (1990). Zinc bioavailability in feed-grade sources of zinc. *Journal of Animal Science* **68**, 684–689.

Wedekind, K. J., Hortin, A. E. & Baker, D. H. (1992). Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate and zinc oxide. *Journal of Animal Science* **70**, 178–187.

Williams, R. J. P. (1984). Zinc: what is its role in biology? *Endeavour* **8**, 65–70.

Williams, R. J. P. (1989). An introduction to the biochemistry of zinc. In *Zinc in Human Biology*, pp. 15–31 [C. F. Mills, editor]. London: Springer-Verlag.

Wu, F. Y.-H. & Wu, C.-W. (1983). The role of zinc in DNA and RNA polymerases. In *Zinc and its Role in Biology and Nutrition* (*Metal Ions in Biological Systems* vol. 15), pp. 157–192 [H. Sigel and A. Sigel, editors]. New York, NY: Marcel Dekker, Inc.

Yasodhara, P., Ramaraju, L. A. & Raman, L. (1991). Trace minerals in pregnancy. 1. Copper and zinc. *Nutrition Research* **11**, 15–21.

Zhou, W., Kornegay, E. T., Lindemann, M. D., Swinkels, J. W. G. M., Welten, M. K. & Wong, E. A. (1994). Stimulation of growth by intravenous injection of copper in weanling pigs. *Journal of Animal Science* (In press).

Printed in Great Britain