PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Absorption, Transport and Tissue Storage of Essential Trace Elements

I. Bremner and C. F. Mills

Phil. Trans. R. Soc. Lond. B 1981 **294**, 75-89
doi: 10.1098/rstb.1981.0090

Phil. Trans. R. Soc. Lond. B **294**, 75–89 (1981)
Printed in Great Britain

Absorption, transport and tissue storage of essential trace elements

By I. Bremner and C. F. Mills

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, U.K.

Intestinal absorption of many essential trace elements probably occurs by saturable and carrier-mediated processes. The nature and efficiency of these are influenced by a range of physiological, nutritional and genetic variables. Special emphasis is given to the influence of exogenous and endogenous ligands of small molecular mass in the intestinal lumen on absorptive efficiency. The effect of enterocyte proteins such as metallothionein which, by sequestering metals, influence their fate during absorption is also considered.

Changes in the metabolic activity of the intestinal mucosa induced by copper or zinc deficiencies influence the fate of other nutrients, either by inhibiting intracellular transport or by preventing the degradation of potential antagonists of absorption.

Conflicting evidence of roles for plasma albumin, transferrin and caeruloplasmin in the transport of zinc and copper is considered.

The extent, location and form in which trace elements are stored in tissues differs between elements and between species. Retention and utilization are also influenced by pregnancy, lactation, stage of foetal development and by genetic variables. Better definition of the effects of these variables would improve the validity of estimates of the trace element requirements of man and other animals.

1. Introduction

Although a low dietary intake of an essential trace element is a frequent cause of deficiency disease, deficiencies often arise when the dietary concentration of the element would normally be considered adequate. Such situations arise when dietary, physiological or genetic variables influence either the absorption or utilization of the element. Some of these effects will be considered in this paper. Using copper and zinc as examples, we shall indicate how knowledge of the mechanisms of absorption and utilization can (i) aid understanding of the aetiology of trace element deficiencies, (ii) indicate how compensatory changes in absorption or utilization occur in response to the supply of trace elements, and (iii) suggest alternative techniques for detecting when trace element supply is suboptimal.

2. Trace element supply and absorption

The efficiency with which trace elements are absorbed and utilized is strongly influenced by many dietary and physiological variables (table 1). Reliable estimation of their effects on trace element metabolism is often the most difficult problem encountered when attempting to determine the adequacy or otherwise of dietary supply (Mills 1979).

(*a*) *Variables influencing intraluminal solubility*

Little is known of the chemical forms in which most essential trace elements occur within the digestive tract or of the influence of this on their availability for absorption. The discovery that virtually all of the soluble copper in grass is present in the form of relatively stable

complexes (Mills 1956*a*, *b*) first focused attention on the possible influence of metal-binding ligands in the food and gastrointestinal secretions on the fate of copper and of other trace metals.

It is well known that the effectiveness with which rumen bacteria form the cobalt complex, cobalamin, profoundly influences the biological availability of cobalt to ruminants. The utilization of other elements is also influenced by processes which modify their form and solubility. Thus, it is probable that the marked decline in efficiency with which dietary copper is utilized by ruminants once rumen microbial activity develops is at least partly attributable to bacterial processes that modify the form and distribution of copper. For example, the cell-wall fraction of rumen bacteria accounts for an appreciable proportion of the copper present in the rumen (Mills 1958). The fate of this component during subsequent digestion is only now being investigated.

Table 1. Variables influencing the efficiency of utilization of trace elements by animals

1. pre-existing tissue reserves
2. anabolic demands:
   (i) growth rate
   (ii) age
   (iii) pregnancy, lactation
3. infection; stress increasing losses
4. genetic variables
5. physical nature of diet (e.g. fluid diets enhancing absorption)
6. chemical composition of diet:
   (i) form and content of essential element
   (ii) presence of ligands enhancing absorption (e.g. in homologous/heterologous milk)
   (iii) presence of organic or inorganic antagonists decreasing absorption or influencing tissue retention (e.g. phytate, cadmium, molybdenum)

The fate of elements that readily form insoluble sulphides, phosphates or hydroxides must also be influenced by microbial activity, by variations in pH within the gastrointestinal tract and by the concentration and metal-binding affinity of ligands secreted into its lumen. Species differences in the effects of inorganic and amino acid sulphur sources on copper utilization reflect the extent to which sulphide is generated as an intermediary metabolite by microorganisms in the digestive tract. In ruminants, the adverse effects of sulphide on both the solubility and absorption of copper from the gut are exacerbated by the presence of molybdenum in the diet. Recent studies suggest that this effect of molybdenum is attributable to the formation, within the rumen, of derivatives related to the tetrathiomolybdates, which, unlike the free sulphide ion, are not readily oxidized in the intestinal lumen and retain a strong affinity for copper (Mills *et al.* 1981*a*, *b*).

The species specificity of the action of molybdenum as an antagonist of copper absorption is related to the fact that little opportunity exists in the digestive tract of the non-ruminant for the generation of sulphide and its subsequent reaction with molybdate. In contrast, when either the tetrathiomolybdate ion or its tungsten analogue is given orally to rats, effects on copper metabolism closely reflect those found in ruminants, i.e. copper absorption is inhibited and, if absorption of the thioanion occurs, systemic inhibitory effects on copper metabolism also become evident. Studies of this interaction have also indicated that antagonistic reactions influencing absorptive efficiency can be very delicately poised. Thus, incomplete replacement of oxygen by sulphur in the series $MoO_4^{2-} \longrightarrow MoS_4^{2-}$ yields oxythiomolybdate derivatives

which, while capable of modifying the tissue distribution of absorbed copper (see later), do not inhibit its absorption (I. Bremner, C. F. Mills & B. W. Young, unpublished results). Recent studies (S. Laurie & N. Clarke, personal communication) suggest that the different physiological effects of the Group VI thioanions may well parallel the stability and insolubility of their [Cu^{I} thioanion] derivatives.

The wide range of antagonistic interactions that can influence trace element absorption adversely has been summarized previously (Mills 1979). Some antagonists also act by reducing intraluminal solubility of the agonist and these are considered in more detail later (§2*f*).

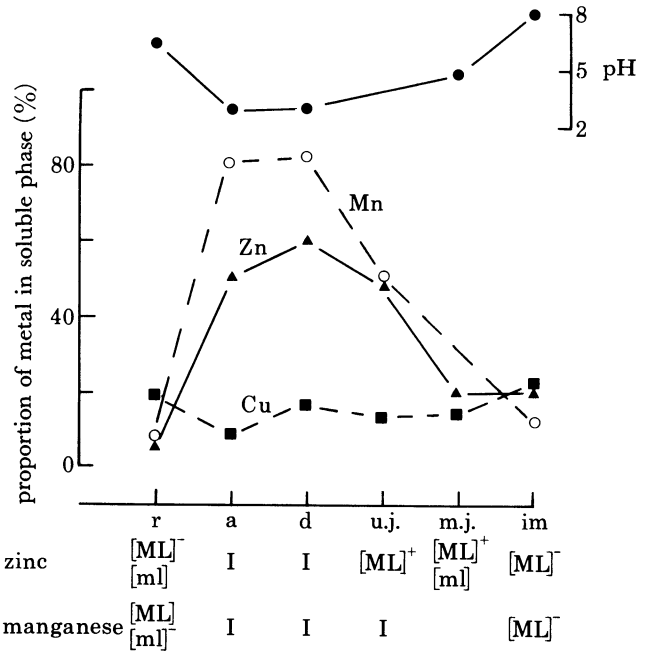

FIGURE 1. Changes in the distribution of copper, zinc and manganese in the alimentary tract of sheep. The proportion of metal present in the soluble phase of lumen contents and the distribution of the principal soluble complexes are shown for different parts of the gut: r, rumen; a, abomasum; d, duodenum; u.j., m.j., upper and mid jejunum; im, ileum. I, ionic form of metal; [ML], neutral complexes; $[ML]^{+}$, cationic complexes; $[ML]^{-}$, anionic complexes. Lower case symbols indicate minor components. The cationic complexes were generally of small molecular mass ($M_r < 1000$) and the anionic complexes of large molecular mass. The low concentrations of soluble Cu precluded identification of Cu-containing species. (From Bremner (1970).)

It is clear that the expression of the potentially adverse effect of such precipitation reactions in the gastrointestinal lumen must be influenced by the simultaneous presence of competing ligands that yield water-soluble complexes. Ligands, such as the amino acids derived from digestion of proteins, can probably act in this way. Thus the inverse relation observed between dietary protein content and the inhibitory effect of phytic acid on zinc absorption by man (Sandstrom *et al.* 1978) may be accounted for by the intra-luminal release of amino acids that partially inhibit formation of the zinc–phytate complex and thus maintain zinc solubility. Studies with pigs and poultry have also shown that some synthetic chelating agents, when administered orally, abolish the adverse effect of dietary phytic acid on zinc availability. In one comprehensive study (Vohra & Kratzer 1964) it was apparent that the magnitude of the protective effect of chelating agents was directly related to the stability constants of the soluble

zinc complexes likely to be formed within the gut lumen. Some of the more stable complexes enhanced both absorption and excretion of zinc.

The influence of intraluminal pH and other variables on the distribution of elements between water-soluble components of gut contents has been demonstrated in studies of the complexes of copper, zinc and manganese in the alimentary tract of sheep (Bremner 1970). Many of the changes in solubility, molecular mass and even the cationic or anionic nature of the complexes were directly related to the pH gradient along the gut (figure 1). Particularly surprising was the finding that the acid environment of the abomasum (true stomach) did not liberate copper from the particulate fractions of digesta and actually decreased the soluble concentrations of copper.

As yet, nothing is known of the availability to the animal of the different copper and zinc complexes that occur in the sheep digestive tract.

(*b*) *Trace element supply and absorptive efficiency*

The efficiency with which dietary sources of copper and zinc are utilized is inversely proportional to their concentrations and to the adequacy of tissue reserves of the metals (see Owen 1964; Weigand & Kirchgessner 1980). Retention of dietary zinc by rats may be as low as 5–10% when dietary zinc is high (more than 200 mg/kg), but 90% or more from zinc-deficient diets (less than 1 mg/kg). Tissue zinc remains constant over a wide range of zinc intakes, indicating that there is efficient homoeostatic control of zinc metabolism. Similar findings have been made with other elements, including copper and manganese.

There is controversy whether copper and zinc homoeostasis is achieved through regulation of absorption or of excretion. This uncertainty reflects the technical difficulties of measuring the precise amount of metal absorbed when a proportion is being returned to the gut in gastrointestinal secretions. Recent studies on zinc utilization (Weigand & Kirchgessner 1980) indicate that both processes are influenced by zinc supply. At very low zinc intakes, young growing rats absorbed almost all their dietary zinc and re-secreted little into the intestine. As dietary zinc content increased, efficiency of absorption progressively decreased but zinc secretion increased. With copper, control is exercised mainly by regulation of the biliary excretion of the metal (Owen 1964), although the efficiency of absorption also varies (Marceau *et al.* 1970; Bremner 1980). The efficiency with which manganese is absorbed by cattle remains almost unchanged over a very wide range of manganese intakes, and homoeostatic control is achieved almost solely by the regulation of biliary excretion (Sansom *et al.* 1978).

(*c*) *Mechanisms and control of zinc absorption*

Transfer of an inorganic element across a membrane frequently involves the binding of the element to an acceptor, its transfer from an aqueous interface across the membrane and, finally, its release (Williams, this symposium). That similar steps are involved in the intestinal absorption of zinc is indicated by kinetic studies on the absorption of the metal from ligated segments of rat intestine (Davies 1980). Two separate phases of zinc absorption were identified, one being rapid and the other slow. Both exhibited saturation kinetics in accord with whole-animal studies, which showed decreased absorptive efficiency at high zinc intakes, suggesting that enzyme-mediated or carrier-mediated processes are involved. The slow phase, and possibly the rapid phase also, was preceded by uptake and accumulation of zinc by the mucosa. Transfer of most of the mucosal zinc to the carcass occurred within 0.5–6.0 h, and it appeared

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

that transfer of the element from mucosa to plasma was the rate-limiting step in the slow phase of zinc absorption.

It has been suggested that a specific ligand in the lumen of the rat intestine is directly involved in the absorption of zinc. This ligand, thought to originate from the pancreas, was found in the intestinal wall and in the milk of certain species (Evans 1980; Lonnerdal *et al.* 1980). Its identity is still uncertain, although it has been claimed to be a prostaglandin (Song & Adham 1979), citrate (Lonnerdal *et al.* 1980) or picolinic acid (Evans 1980). Picolinic acid enhances the absorption of zinc by rats (Evans & Johnson 1980), but this property is shared by many other chelating agents. It would be surprising if the effects of picolinic acid were unique when many other potential ligands are also present in the mucosal environment. Other studies throw doubt on claims that ligands secreted from the pancreas play a role in zinc absorption (Davies 1980) and emphasize the difficulty of preventing the appearance of artefacts when attempts are made to isolate zinc-binding ligands from the intestinal mucosa (Cousins 1979).

It is possible that sequestration of zinc as Zn-metallothionein within enterocytes may also influence the efficiency with which Zn is absorbed. Oral or parenteral administration of zinc induces its synthesis in the mucosa of rats (Cousins 1979; Hall *et al.* 1979) but, in contrast, little zinc is present in this form in zinc deficient rats. Diversion of any excess of absorbed zinc into this relatively inert mucosal metalloprotein appears to account for the reduced efficiency with which large oral doses of zinc are absorbed.

Most studies on the involvement of metallothionein in the control of zinc absorption have been made with animals given relatively large amounts of zinc orally or parenterally, and there is some doubt as to the importance of metallothionein in controlling zinc absorption at physiological zinc intakes. For example, in one experiment with rats, significant increases in mucosal zinc-metallothionein concentrations only occurred when dietary zinc intake was more than 450 mg/kg, i.e. nearly 10 times normal (Hall *et al.* 1979). The possibility cannot therefore be excluded that synthesis of this protein is simply a response to increased intracellular zinc concentrations arising from the swamping of other control mechanisms.

The identity of the carriers and of the exchange reactions involved in the transmucosal flux of selenium, molybdenum and manganese is unknown. For elements such as selenium, which may be presented to the intestinal mucosa in anionic forms (i.e. as selenite or selenate), or as analogues of sulphur amino acids, there is evidence for the existence of more than one absorptive mechanism.

(*d*) *Effects of pregnancy and stress*

Evidence that trace element absorption is partly regulated by demand is supported by studies on zinc absorption by pregnant and lactating rats (Davies & Williams 1977). The quantity of zinc absorbed by isolated loops of duodenum increased by up to 80% in the late stages of gestation, but not at earlier stages (12–15 days), when foetal demand for zinc was less. Copper absorption is also enhanced in pregnant rats, although this is related neither to foetal demand (Davies & Williams 1976) nor to changes in the levels of female sex hormones, since administration of oestrogens to rats decreases copper absorption (Cohen *et al.* 1979). The probability that absorptive efficiency increases in response to the high demands of pregnancy and lactation has not been taken into account when estimates of the trace element requirements of man and for animals other than rats have been derived.

Zinc absorption is also influenced by circulating glucocorticoid hormones. Injection of hydrocortisone into chicks increased zinc absorption and retention by 50% (Sas & Bremner

1979). Administration of endotoxin and of leucocytic endogenous mediator elicited a similar response in rats and chicks (Pekarek & Evans 1975, 1976; Sas & Bremner 1979), indicating that enhanced zinc absorption could be a general response to acute stress and infection. Even relatively mild stress, as induced by unusual physical exercise, enhances zinc absorption in rats (Starcher *et al.* 1980). Shock, infection and trauma are all associated with increased permeability of the gut to macromolecules, and this may be relevant to the effects on zinc absorption described above.

(*e*) *Trace element absorption by neonatal animals*

The permeability of the gut of neonatal animals to macromolecules is almost certainly responsible for enhanced absorption of both essential and non-essential elements. Studies by Mistilis & Mearrick (1969) and by Mann *et al.* (1979) suggest that pinocytic absorption of both copper and iron may predominate in the neonatal rodent. However, species differences in the rate at which the gut becomes impermeable to macromolecules suggest that this route of trace metal absorption may not be generally significant. Furthermore, there is no evidence that elements absorbed by this process have the same biopotency as those absorbed by routes over which homoeostatic control is normally exercised (Mills & Davies 1979).

(*f*) *Effects of dietary composition on trace element absorption*

Trace element absorption can also be influenced greatly by organic and inorganic dietary components. The physicochemical processes involved are poorly understood and data on the quantitative aspects of the interactions between trace elements and their antagonists are scarce. A typical example is provided by the antagonistic effect of dietary phytate on zinc availability. Phytates present in cereals and some vegetable sources of protein have frequently been shown to be responsible for the development of zinc deficiency in simple-stomached species and have been implicated in the aetiology of zinc deficiency in man. While the principal effect of phytate is to decrease the solubility of zinc in the lumen of the gut and so decrease zinc absorption, it also inhibits the reabsorption of zinc secreted into the gut (Davies & Nightingale 1975). Increases in dietary calcium enhance the antagonistic effect of phytate, probably through formation in the gut of an insoluble ternary zinc–calcium–phytate complex. Little is known of the structure of this complex or of the factors that influence its formation or stability. These could include the relative concentrations of zinc, calcium and phytate and of naturally occurring zinc-binding ligands in the lumen of the gut.

Studies with experimental animals indicate that the phytate content of certain human foods could be such as to inhibit zinc utilization (Davies & Reid 1979). Whether the relatively low calcium content of human diets potentiates the effect of phytate is not known. The antagonistic effects of phytate on trace element metabolism in man may not be confined to zinc; phytate can also reduce the retention of copper and manganese in rats (Davies & Nightingale 1975).

As shown in table 2, the absorption of many trace metals is influenced by the dietary concentrations of other inorganic elements. In many cases, close structural similarities between the competing elements or ions suggest that isomorphous substitutions within carrier systems may be the basis of their mutual antagonism. Thus, the inhibitory effects of sulphate and tungstate on molybdate absorption arise from competition between the anions for a common carrier in the intestinal mucosa (Cardin & Mason 1975). The adverse effects of cadmium on zinc absorption may have a similar origin, since cadmium inhibits the uptake and vascular

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

transfer of zinc by perfused mouse duodenum (Hamilton *et al.* 1978). This effect is only evident, however, in iron-deficient animals. Iron status also influences zinc absorption; it is enhanced by iron deficiency and reduced by increases in dietary iron. Mild iron deficiency also enhances the absorption of cobalt, manganese and lead. Since iron deficiency is one of the commonest nutritional disorders, such findings could have particular relevance to man.

The induction of metallothionein synthesis in the intestinal mucosa by high dietary intakes of zinc and cadmium has secondary effects on the absorption of copper and may induce copper deficiency (Van Campen & Scaife 1967; Hall *et al.* 1979). When rats received diets containing 30 or 900 mg Zn/kg, the decrease in the absorption of an oral dose of ^{64}Cu in zinc-supplemented animals could be correlated with increased binding of ^{64}Cu in the mucosa, mainly in the form of ^{64}Cu-metallothionein (figure 2) (Hall *et al.* 1979). Lower dietary zinc concentrations (150 or 450 mg/kg) affected neither ^{64}Cu absorption nor intestinal metallothionein concentrations significantly.

TABLE 2. INORGANIC ANTAGONISMS INFLUENCING ABSORPTION OR METABOLISM OF SOME ESSENTIAL TRACE ELEMENTS

agonist	*antagonist*
copper	zinc, cadmium, sulphides, thiomolybdate derivatives, iron
zinc	cadmium, copper, iron, calcium, lead, phosphates
selenium	specific organic and inorganic sulphur analogues of dietary Se sources, cadmium, arsenic, silver
molybdenum	sulphur and tungsten analogues of dietary Mo sources
manganese	iron, copper, phosphates

There are other grounds for suggesting that intestinal metallothionein may be involved, directly or indirectly, in processes that regulate copper absorption. Thus, there is an inverse relation between copper absorption and the intestinal Cu-metallothionein content in (i) rats dosed with oestrogen (Cohen *et al.* 1979), (ii) human infants with copper deficiency induced by genetic defects in copper metabolism (Menkes's disease), and (iii) brindled mice with a similar genetic defect (Danks 1977; Evans & Reis 1978; Bremner 1980). There is, however, no convincing evidence that decreases in the efficiency of copper absorption that occur when copper intake is high are mediated by increased metallothionein production in the intestine.

(*g*) *Trace element deficiency and the integrity of the intestinal mucosa*

Both the metabolic activity and the structural integrity of the intestinal mucosa can be influenced by essential trace element deficiency. Extensive structural defects develop in the enterocyte mitochondria of copper deficient cattle and are accompanied by a marked loss of mucosal cytochrome oxidase activity (Fell *et al.* 1975) and, later, by villus atrophy (Mills *et al.* 1976). The severity of cytological damage and the sensitivity of mucosal cytochrome oxidase to copper depletion and repletion suggest that the intestine may be particularly sensitive to copper deficiency. It is certainly one of several tissues in which the development of copper deficiency is accompanied by secondary defects in the absorption and transport of iron (Lee *et al.* 1968). While the metabolic origin of these defects is a subject of controversy (Mills 1980), they lead to the accumulation of histochemically demonstrable deposits of iron, to a delay in iron absorption and to a decrease in total body iron content.

Other evidence indicates that metabolic defects that arise in the mucosa as a result of trace element deficiency can accelerate the development of a deficiency state. Davies *et al.* (1975,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

1978) investigated the action of phytate as an antagonist of zinc absorption and found that the induced deficiency of zinc resulted in a significant decline in the activity of intestinal phytase. Thus the syndrome induced by excessive dietary phytate included an impaired ability to degrade the antagonist that first provoked its development!

These findings and other, largely circumstantial, evidence that deficiencies of copper and cobalt may impair the immune response of the intestinal mucosa suggest that more detailed studies of the effects of deficiency on intestinal mucosal function should be made.

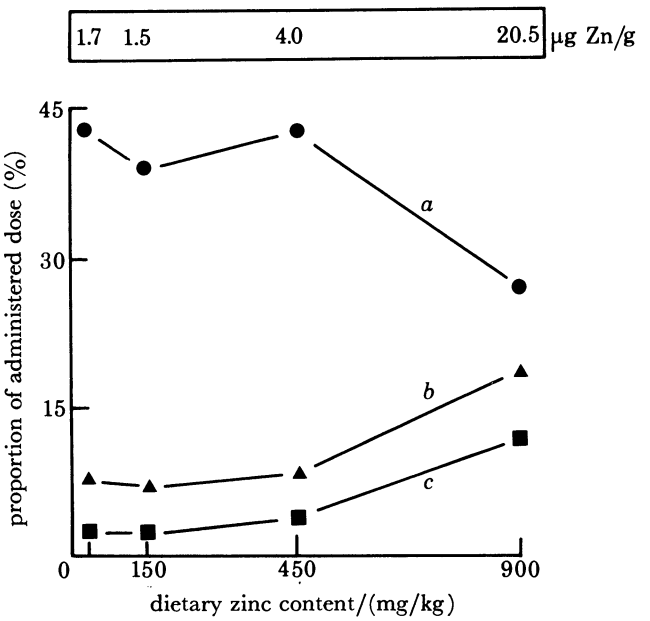

FIGURE 2. Effect of zinc supplementation on the absorption of ^{64}Cu by rats. (*a*) ^{64}Cu absorption; (*b*) ^{64}Cu in mucosa of small intestine; (*c*) ^{64}Cu present in mucosa as metallothionein [MT]; inset, concentration of zinc present as intestinal metallothionein. The values for mucosal ^{64}Cu are based on the assumption that the total mass of mucosa was about 5 g. (From Hall *et al.* (1979).)

3. TRANSPORT OF TRACE ELEMENTS

Although the predictive validity of models describing the likely sequence of events governing trace metal release from the basolateral membrane of the intestine into plasma has never been adequately assessed, some qualitative data are now becoming available for zinc and copper. Studies with isolated perfused rat intestine (Smith & Cousins 1980) indicate that zinc is bound to albumin as it leaves the serosal surface of the gut wall, and not to transferrin as has been suggested (Evans 1976). If this is so, it seems unlikely that the efficiency of zinc absorption could ever be limited under normal physiological conditions by the inadequate flux of binding sites available on the protein. The molar ratio of zinc to albumin in plasma is about 0.06 under normal conditions. Even allowing for only one zinc-binding site per albumin molecule, there would always be an excess of sites available for zinc binding.

The uptake of copper from the intestine and its immediate transport to the liver and other tissues is also thought to involve albumin as the transport protein, although amino acids such as histidine, threonine and glutamine may also be involved. As with zinc, the transport of plasma albumin through intestinal tissues is unlikely to be a rate-limiting factor influencing the

transfer of copper to tissues. Variables influencing the rate of release of copper from albumin may have greater significance. Thus, it has been suggested that the absence of the specific copper-binding site on albumin (Appleton & Sarkar 1971) may be responsible for the abnormally rapid rate of clearance of copper from plasma and the enhanced hepatic uptake of copper in the dog (Goresky *et al.* 1968).

The release of copper from plasma albumin is also affected in ruminant animals receiving diets with a high molybdenum content. Such animals have increased plasma copper concentrations (Smith *et al.* 1968; Bremner & Young 1978), which probably arise from a decreased

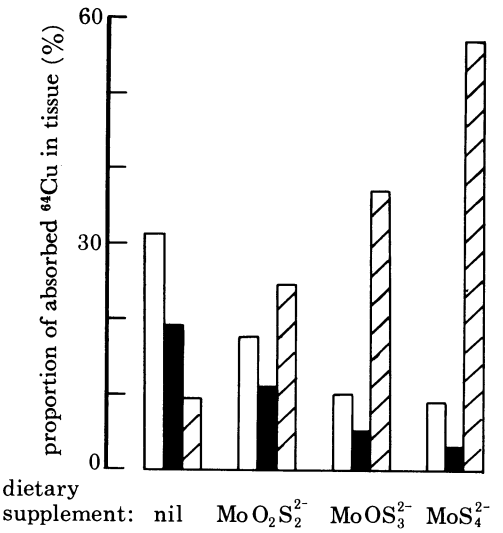

FIGURE 3. The effects of dietary supplementation with $(NH_4)_2MoO_2S_2$, $(NH_4)_2MoOS_3$ and $(NH_4)_2MoS_4$ on the tissue distribution of absorbed ^{64}Cu in rats. Rats were given a diet containing 3 mg Cu/kg and were killed 4 h after receiving an oral dose of ^{64}Cu. Results are given for liver (□), kidneys (■) and plasma (▨). (I. Bremner, C. F. Mills & B. W. Young, unpublished observations.)

rate of clearance of copper from the plasma (Smith *et al.* 1968). This, in turn, appears to be a consequence of the formation in the rumen of thiomolybdates, or related compounds which, when absorbed, enhance the affinity of albumin for copper (Mills & Bremner 1980). This view is supported by the changes which occur in the clearance of ^{64}Cu from the plasma of rats given di-, tri- and tetrathiomolybdates in their diet. Furthermore, such treatment influences the tissue distribution of orally administered ^{64}Cu, its retention by plasma being enhanced strongly at the expense of liver and kidney (figure 3). Progressively increasing substitution of the oxythiomolybdates with sulphido groups increases the magnitude of these anomalies of copper distribution which are believed to reflect a direct reaction of copper with the thio- or oxythiomolybdates retained by the albumin fraction of plasma.

Although it is generally accepted that albumin is involved in the transport of copper from the intestines to the liver, there is evidence that caeruloplasmin may play an important role in its subsequent transport to other tissues for incorporation into enzymes such as cytochrome oxidase, superoxide dismutase and lysyl oxidase (Marceau & Aspin 1973; Frieden 1979; Harris *et al.* 1980). For example, the restoration of cytochrome oxidase activity in several tissues of copper-deficient rats was much faster in animals injected with caeruloplasmin than when they were given equimolar doses of copper–albumin or the copper–histidine complex

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(Frieden 1979). Although caeruloplasmin may facilitate the transfer of copper to the sites of synthesis of the above enzymes, it is not known whether this involves the direct entry of caeruloplasmin into the cell and the donation of its copper to the respective apoenzymes, or whether copper is first reduced and incorporated into receptors at cell membranes (Frieden 1979). However, the possibility cannot be excluded that the apparently more effective utilization of caeruloplasmin copper results from the faster removal of the other forms of copper by the liver. Resolution of this problem must await studies with cell cultures. If caeruloplasmin has a role as a copper carrier, it will be important to establish whether the changes in caeruloplasmin concentration in protein deficiency, during stress, pregnancy and after the administration of oral contraceptives induce other changes in copper metabolism.

4. Storage of trace elements

The period for which a reduced intake of an essential trace element can be tolerated is influenced by the extent to which stores of the element have accumulated previously and by the ease with which they can be mobilized. The tissue distribution and physiological availability of such reserves differs between species and, within a species, both are influenced by genetic variables and age.

Marked differences exist between elements in the extent to which any body reserve is concentrated within a single organ or distributed widely between tissues. Thus, no locally concentrated store of chromium, selenium or manganese has been identified. Zinc reserves are usually small and located mainly in the skeleton and muscle mass, although appreciable hepatic accumulation of zinc can also occur in the liver of foetal animals (Bremner *et al.* 1977). Mobilization of the skeletal reserve of zinc is influenced by calcium status and is enhanced by conditions that promote bone resorption (Tao & Hurley 1975).

Pregnancy and lactation also influence the rates of accretion and mobilization of tissue reserves. Pregnant rats accumulate copper throughout gestation, and much of this copper is utilized during subsequent lactation (Williams *et al.* 1977). Thus, pregnancy increased by 60% the amount of copper in the maternal carcass (excluding the products of conception), but body copper had returned to normal by the 14th day post-partum. If all this copper were transferred to their sucking offspring, it would have met about 25% of their demand for copper. The need for such a reserve is indicated by the observation that the lactating rat transfers to her offspring more copper than she can absorb from her own diet. The site of copper deposition was not established in these experiments, but the metal was probably distributed throughout the body, since there was no major increase in the copper content of the femur, kidneys or, most surprisingly, the liver.

(*a*) *Metallothionein and the hepatic accumulation of copper*

The liver is commonly regarded as the major storage organ for copper and considerable attention has thus been paid to the forms in which it is retained.

There are rapid changes in the distribution of ^{64}Cu in the liver of rats injected with tracer quantities of ^{64}Cu (Marceau & Aspin 1973). Much is initially associated with metallothionein (Bremner 1978), but is then transferred rapidly to other hepatic proteins, including superoxide dismutase.

When rats are injected with large quantities (100–300 μg) of non-radioactive copper, the

pattern of copper retention differs. Maximum concentrations of copper-metallothionein are not achieved until after about 10 h, and the protein persists in the liver for much longer, the half-life being about 17 h (Bremner *et al.* 1978). Unlike the tracer studies referred to above, in which the binding of ^{64}Cu to metallothionein could have occurred by exchange reactions, the incorporation of non-radioactive copper into the protein is clearly a consequence of synthesis *de novo* of the protein. Thus the incorporation of [^{35}S]cysteine into this sulphur-rich protein is stimulated by the administration of copper (Bremner *et al.* 1978) and is inhibited by actinomycin D (Premakumar *et al.* 1975), suggesting that the induction of metallothionein synthesis is under transcriptional control. The administration of cycloheximide also blocks the Cu-metallothionein synthesis but does not prevent the uptake of copper by the liver (Bremner 1978). Similar observations have been made with primary monolayer cultures of rat liver parenchymal cells (Weiner & Cousins 1980). Thus the hepatic uptake of copper is not dependent on the ability to synthesize metallothionein. In contrast, the inhibition of protein synthesis clearly prevents the hepatic uptake of zinc (Richards & Cousins 1975).

Metallothionein is also a major copper-binding protein in the liver of animals receiving copper in the diet rather than parenterally. Up to 40% of the copper in pig liver can be present in this form, the concentration of the protein being directly related to the liver copper content (Bremner 1978). Since concentrations of the protein respond readily to changes in the copper status of the animal, it could be argued that metallothionein has a temporary or short-term storage function for copper. However, it cannot be essential for the utilization of hepatic copper, since there are no major disturbances in copper metabolism in zinc-deficient animals, in which hepatic accumulation of copper-metallothionein does not occur (Bremner 1978).

(*b*) *Copper accumulation in foetal livers*

Copper-metallothionein is frequently present in large amounts in the liver of foetal or neonatal animals, which often have exceptionally high liver copper contents (Hartmann & Weser 1977; Riordan & Richards 1980). However, the distribution of the copper-rich protein within these livers differs from that in livers of adult animals; it is located principally in the particulate fractions of the liver, and not in the cytosol (Riordan & Richards 1980). The location of the copper-rich metallothionein in foetal livers has not been established, but it is probable that it occurs in the lysosomes, since the insoluble copper-protein, neonatal mitochondrocuprein, found in the lysosomes of newborn calves is a polymerized form of metallothionein (Porter 1974).

About half the total body copper in the developing foetus is usually present in the liver (Williams *et al.* 1978). It is often assumed that this provides the newborn animal with a readily available reserve at a time when the intake of copper from milk is low. However, the amount that is used for metabolic purposes has not been established. Recent studies on the rate of loss of copper from the liver of newly weaned calves suggest that much of the hepatic copper may be lost by endogenous secretions (McDonald *et al.* 1979). When calves received a copper-deficient diet after weaning, the rate of loss of copper from the liver was related to liver copper concentration by the equation

$$[\mathrm{Cu}]_t = [\mathrm{Cu}]_0 \mathrm{e}^{-kt},$$

where $[\mathrm{Cu}]_0$ and $[\mathrm{Cu}]_t$ are the liver copper concentrations (milligrams per kilogram dry mass) at the start of the depletion and after t days respectively. The value of the exponent k, 0.027, indicates that the half-life of copper in the liver of these animals was about 27 days and did

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

not differ significantly between three separate studies. The amount of copper lost from the liver of many calves exceeded their estimated requirement for growth and was probably excreted in bile. These findings support the view of Evans (1973) that the accumulation of copper in the liver of newborn animals could sometimes be the result of a limited capacity for excretion of copper rather than a need to form copper reserves during foetal development and in the neonatal period.

5. Conclusions

Knowledge of the processes involved in the absorption, transport and excretion of trace elements is very incomplete. It has been established that the efficiency of absorptive and of excretory processes responds to changes in trace element demand, but the nature of the 'trigger' mechanism is obscure. There is some evidence that, in neonatal animals, the absorption of some trace elements may occur by pinocytosis, but whether there is any mechanism for the pericellular transfer of metals through the so-called 'tight junctions' in the intestinal wall that might account for the marked increase in absorptive efficiency that occurs when liquid diets are given has not been studied. Similarly, the possibility that lipid-soluble or phospholipid derivatives of the trace metals in the digestive tract may be able to bypass control mechanisms that normally regulate metal absorption in the intestine has not been investigated.

Changes in dietary composition have marked effects on the availability of trace elements, but the quantitative aspects of most of these relations have still to be defined. Most studies have involved the use of diets containing excessive amounts of antagonists, and it is usually impossible to extrapolate the findings to more 'normal' situations, where there are only moderate increases in the concentration of the antagonist. The mechanisms whereby interaction occurs has rarely been established in detail. For example, the mucosal binding sites involved in the uptake and transfer of both iron and zinc have not been identified. The fate of copper after its incorporation into zinc-induced mucosal metallothionein is still a matter of conjecture.

Considerable effort is being expended on the study of the physiological role of metallothionein and its involvement in the metabolism of both essential and non-essential elements. The wide distribution of the protein in most animal tissues, in plants, marine organisms and micro-organisms, and the fact that several metals can induce synthesis of the protein, suggest a fundamental role for the protein in metal metabolism, possibly in storage or detoxication. However, the lack of discrimination between metals could also argue against a role in the maintenance of homoeostasis. It may be that synthesis of the protein is merely a short-term response to increased intracellular concentrations of a metal, with its subsequent fate, and therefore the function of the protein, being determined by the immediate needs of the animal.

This does not imply that studies on the binding of metals to metallothionein are physiologically irrelevant but rather that we have not yet defined, in sufficient detail, the kinetic relations influencing the distribution of metal between this and other proteins involved in absorption and tissue retention of metals. There is already evidence that susceptibility to copper toxicity is reflected by changes in such relations. Thus sheep, a species particularly susceptible to copper poisoning, appear to be unable to sequester excess liver copper as Cu-metallothionein. Instead, the excess accumulates in a protein-containing and sulphur-rich particulate fraction, the extent and distribution of which appears to be related to the severity of hepatic dysfunction.

Better understanding of the primary, genetically determined, defects of trace element metabolism in diseases such as acrodermatitis enteropathica and Menkes's disease will un-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

doubtedly require elucidation of the forms in which zinc and copper occur in tissues. The latter disease is particularly interesting because clinical signs of copper deficiency are accompanied by enhanced concentrations of copper in tissues such as the intestines and kidneys. Investigation of the reasons for such anomalies in the distribution and mobility of copper could well contribute to the resolution of other difficulties in interpreting data on tissue copper content. These include the problem of distinguishing clinically normal animals from those with covert or overt signs of deficiency when analysis of tissues from both reveals a low content of copper.

The influence of genetic variables on trace element metabolism and distribution in tissues is not restricted to these rare X-linked recessive disorders. Wiener (1979) has reported that marked differences exist in plasma and liver copper content between breeds of sheep, and that susceptibility to copper deficiency or excess also differs between breeds. Other studies have shown that the intracellular distribution of copper in hepatocytes differs markedly between strains of rats. There is no reason to assume that such effects are restricted to sheep, to rats or to copper metabolism.

This account, although largely confined to consideration of zinc and copper, has indicated the frequency with which inadequate understanding of the processes of trace element absorption, transport and storage so frequently hinders our ability to anticipate the effect of variables that influence trace element 'availability'. The paucity of kinetic studies of these processes has been particularly emphasized. Until such information becomes available, it is unrealistic to expect that future attempts to define the trace element requirements of man and animals will overcome the most important limitation of existing estimates: a lack of flexibility that hinders extrapolation to subjects differing in stage of physiological development, diet and genetic background.

References (Bremner & Mills)

Appleton, D. W. & Sarkar, B. 1971 The absence of specific copper-(II)-binding site in dog albumin. *J. biol. Chem.* **246**, 5040–5046.

Bremner, I. 1970 Zinc, copper and manganese in the alimentary tract of sheep. *Br. J. Nutr.* **24**, 769–783.

Bremner, I. 1978 Factors influencing the occurrence of copper-thioneins in tissues. In *Metallothionein* (ed. J. H. R. Kagi & M. Nordberg), pp. 273–280. Basel: Birkhauser.

Bremner, I. 1980 Absorption, transport and storage of copper. In *Biological roles of copper* (*Ciba Foundation Symposium* no. 79), pp. 23–48. Amsterdam: Excerpta Medica.

Bremner, I. & Young, B. W. 1978 Effect of dietary molybdenum and sulphur on the distribution of copper in plasma and kidneys of sheep. *Br. J. Nutr.* **39**, 325–336.

Bremner, I., Hoekstra, W. G., Davies, N. T. & Young, B. W. 1978 Effect of zinc status of rats on the synthesis and degradation of copper-induced metallothionein. *Biochem. J.* **174**, 883–892.

Bremner, I., Williams, R. B. & Young, B. W. 1977 Distribution of copper and zinc in the liver of the developing sheep foetus. *Br. J. Nutr.* **38**, 87–92.

Cardin, C. J. & Mason, J. 1975 Sulphate transport by rat ileum. *Biochim. biophys. Acta* **394**, 46–54.

Cohen, D. I., Illowsky, B. & Linder, M. C. 1979 Altered copper absorption in tumour-bearing and estrogen-treated rats. *Am. J. Physiol.* **236**, E309–E315.

Cousins, R. J. 1979 Regulation of zinc absorption: role of intracellular ligands. *Am. J. clin. Nutr.* **32**, 339–345.

Danks, D. M. 1977 Copper transport and utilization in Menkes' syndrome and in mottled mice. *Inorg. Perspect. Biol. Med.* **1**, 73–100.

Davies, N. T. 1980 Studies on the absorption of zinc by rat intestine. *Br. J. Nutr.* **43**, 189–203.

Davies, N. T. & Flett, A. A. 1978 The similarity between alkaline phosphatase (EC 3.1.3.1) and phytase (EC 3.1.3.8) activities in rat intestine and their importance in phytate-induced zinc deficiency. *Br. J. Nutr.* **39**, 307–316.

Davies, N. T. & Nightingale, R. 1975 The effects of phytate on intestinal absorption and secretion of zinc. *Br. J. Nutr.* **34**, 243–258.

Davies, N. T. & Reid, H. 1979 Phytate and trace element contents of TVPs. *Br. J. Nutr.* **41**, 579–589.

Davies, N. T. & Williams, R. B. 1976 The effects of pregnancy on uptake and distribution of copper in the rat. *Proc. Nutr. Soc.* **35**, 4A.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Davies, N. T. & Williams, R. B. 1977 The effect of pregnancy and lactation on the absorption of zinc. *Br. J. Nutr.* **38**, 417–423.
Evans, G. W. 1973 Copper homeostasis in the mammalian system. *Physiol. Rev.* **53**, 535–570.
Evans, G. W. 1976 Transferrin function in zinc absorption and transport. *Proc. Soc. exp. Biol. Med.* **151**, 775–778.
Evans, G. W. 1980 Normal and abnormal zinc absorption in man and animals: the tryptophan connection. *Nutr. Rev.* **38**, 137–141.
Evans, G. W. & Johnson, E. C. 1980 Zinc concentration of liver and kidney from rat pups nursing dams fed supplemental zinc dipicolinate or zinc acetate. *J. Nutr.* **110**, 2121–2124.
Evans, G. W. & Reis, B. O. 1978 Impaired copper homeostasis in neonatal male and adult female brindled mice. *J. Nutr.* **108**, 554–560.
Fell, B. F., Dinsdale, D. & Mills, C. F. 1975 Changes in enterocyte mitochondria associated with deficiency of copper. *Res. vet. Sci.* **18**, 274–281.
Frieden, E. 1979 Caeruloplasmin: the serum copper transport protein with oxidase activity. In *Copper in the environment* (ed. J. O. Nriagu), vol. 2, pp. 241–284. New York: Wiley Interscience.
Goresky, C. A., Holmes, T. H. & Sass-Kortsak, A. 1968 Initial uptake of copper by the liver in the dog. *Can. J. Physiol. Pharmac.* **46**, 771–784.
Hall, A. C., Young, B. W. & Bremner, I. 1979 Intestinal metallothionein and the mutual antagonism between copper and zinc in the rat. *J. inorg. Biochem.* **11**, 57–66.
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. *Can. J. Physiol. Pharmac.* **56**, 384–389.
Harris, E. D., Rayton, J. K., Balthrop, J. E., DiSilvestro, R. A. & Garcia-de-Quevedo, M. 1980 Copper and the synthesis of elastin and collagen. In *Biological roles of copper* (*Ciba Foundation Symposium* no. 79), pp. 163–182. Amsterdam: Excerpta Medica.
Hartmann, H.-J. & Weser, U. 1977 Copper-thionein from fetal bovine liver. *Biochim. biophys. Acta* **491**, 211–222.
Lee, G. L., Nacht, S., Lukens, J. N. & Cartwright, G. E. 1968 Iron metabolism in copper deficient swine. *J. clin. Invest.* **47**, 2058–2069.
Lonnerdal, B., Stanislowski, A. G. & Hurley, L. S. 1980 Isolation of a low molecular weight zinc binding ligand from human milk. *J. inorg. Biochem.* **12**, 71–78.
Mann, J. R., Camakaris, J. & Danks, D. M. 1979 Copper metabolism in mottled mouse mutants. *Biochem. J.* **180**, 613–619.
Marceau, N. & Aspin, N. 1973 The intracellular distribution of radio copper derived from caeruloplasmin and from albumin. *Biochim. biophys. Acta* **293**, 338–350.
Marceau, N., Aspin, N. & Sass-Kortsak, A. 1970 Absorption of copper-64 from gastrointestinal tract of the rat. *Am. J. Physiol.* **218**, 377–383.
McDonald, I., Mills, C. F., Dalgarno, A. C. & Simpson, A. M. 1979 Rates of loss of hepatic copper during copper-depletion of cattle. *Proc. Nutr. Soc.* **38**, 59A.
Mills, C. F. 1956*a* Avidity of the water-soluble components of herbage for added cupric ions. *Nature, Lond.* **177**, 793–794.
Mills, C. F. 1956*b* Studies of the copper compounds in aqueous extracts of herbage. *Biochem. J.* **63**, 187–190.
Mills, C. F. 1958 Comparative metabolic studies of inorganic and complex forms of copper in rats and sheep. *Soil Sci.* **85**, 100–105.
Mills, C. F. 1979 Trace elements in animals. *Phil. Trans. R. Soc. Lond.* B **288**, 51–63.
Mills, C. F. 1980 Metabolic interactions of copper with other trace elements. In *Biological roles of copper* (*Ciba Foundation Symposium* no. 79), pp. 49–69. Amsterdam: Excerpta Medica.
Mills, C. F. & Bremner, I. 1980 Nutritional aspects of molybdenum in animals. In *Molybdenum and molybdenum-containing enzymes* (ed. M. Coughlan), pp. 519–542. Oxford: Pergamon Press.
Mills, C. F. & Davies, N. T. 1979 Perinatal changes in the absorption of trace elements. In *Development of mammalian absorptive processes* (*Ciba Foundation Symposium* no. 70), pp. 247–258. Amsterdam: Excerpta Medica.
Mills, C. F., Dalgarno, A. C. & Wenham, G. 1976 Biochemical and pathological changes in tissues of Friesian cattle during the experimental induction of copper deficiency. *Br. J. Nutr.* **35**, 309–311.
Mills, C. F., El-Gallad, T. T. & Bremner, I. 1981*a* Effects of molybdate, sulfide and tetrathiomolybdates on copper metabolism in rats. *J. inorg. Biochem.* (In the press.)
Mills, C. F., El-Gallad, T. T., Bremner, I. & Wenham, G. 1981*b* Copper and molybdenum absorption by rats given ammonium tetrathiomolybdate. *J. inorg. Biochem.* (In the press.)
Mistilis, S. P. & Mearrick, P. T. 1969 The absorption of ionic, biliary and plasma radiocopper in neonatal rats. *Scand. J. Gastroenterol.* **4**, 691–696.
Owen, C. A. 1964 Absorption and excretion of ^{64}Cu-labelled copper by the rat. *Am. J. Physiol.* **207**, 1203–1206.
Pekarek, R. S. & Evans, G. W. 1975 Effect of acute infection and endotoxemia on zinc absorption in the rat. *Proc. Soc. exp. Biol. Med.* **150**, 755–758.
Pekarek, R. S. & Evans, G. W. 1976 Effect of leucocytic endogenous mediator (LEM) on zinc absorption in the rat. *Proc. Soc. exp. Biol. Med.* **152**, 573–575.
Porter, H. 1974 The particulate half-cystine-rich copper protein of newborn liver. *Biochem. biophys. Res. Commun.* **56**, 661–668.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Premakumar, R., Winge, D. R., Wiley, R. D. & Rajagopalan, K. V. 1975 Copper-induced synthesis of copper-chelatin in rat liver. *Archs Biochem. Biophys.* **170**, 267–277.

Richards, M. P. & Cousins, R. J. 1975 Influence of parenteral zinc and actinomycin D on tissue zinc uptake and synthesis of a zinc-binding protein. *Bioinorg. Chem.* **4**, 215–224.

Riordan, J. R. & Richards, V. 1980 Human fetal liver contains both zinc- and copper-rich forms of metallothionein. *J. biol. Chem.* **255**, 5380–5383.

Sandstrom, B., Arvidsson, B., Bjorn-Rasmussen, E. & Cederblad, A. 1978 Zinc absorption from bread meals. In *Trace element metabolism in man and animals*-3 (ed. M. Kirchgessner), pp. 129–131. Freising-Weihenstephan: Arbeitskreis fur Tierernahrungsforschung Weinhenstephan.

Sansom, B. F., Symonds, H. W. & Vagg, M. J. 1978 The absorption of dietary manganese by dairy cows. *Res. vet. Sci.* **24**, 366–369.

Sas, B. & Bremner, I. 1979 Effect of acute stress on the absorption of zinc by the chick. *J. inorg. Biochem.* **11**, 67–76.

Smith, B. S. W., Field, A. C. & Suttle, N. F. 1968 Effect of copper, molybdenum and sulphate on copper metabolism in the sheep. *J. comp. Path.* **78**, 449–461.

Smith, K. T. & Cousins, R. J. 1980 Quantitative aspects of zinc absorption by isolated, vascularly perfused rat intestine. *J. Nutr.* **110**, 316–323.

Song, M. K. & Adham, N. F. 1979 Evidence for an important role of prostaglandins E_2 and F_2 in the regulation of zinc transport in the rat. *J. Nutr.* **109**, 2152–2159.

Starcher, B. C., Glauber, J. G. & Madaras, J. G. 1980 Zinc absorption and its relationship to intestinal metallothionein. *J. Nutr.* **110**, 1391–1397.

Tao, S.-H. & Hurley, L. S. 1975 Effect of dietary calcium deficiency during pregnancy on zinc mobilization. *J. Nutr.* **105**, 220–225.

Van Campen, D. R. & Scaife, P. U. 1967 Zinc interference with copper absorption in rats. *J. Nutr.* **91**, 473–476.

Vohra, P. & Kratzer, F. H. 1964 Influence of various chelating agents on the availability of zinc. *J. Nutr.* **82**, 249–256.

Weigand, E. & Kirchgessner, M. 1980 Total true efficiency of zinc utilization. *J. Nutr.* **110**, 469–480.

Weiner, A. L. & Cousins, R. J. 1980 Copper accumulation and metabolism in primary monolayer cultures of rat liver parenchymal cells. *Biochim. biophys. Acta* **629**, 113–125.

Wiener, G. 1979 Review of genetic aspects of mineral metabolism. *Livestock Prod. Sci.* **6**, 223–232.

Williams, R. B., Davies, N. T. & McDonald, I. 1977 The effects of pregnancy and lactation on copper and zinc retention in the rat. *Br. J. Nutr.* **38**, 407–416.

Williams, R. B., McDonald, I. & Bremner, I. 1978 The accretion of copper and zinc by the foetuses of the prolific ewe. *Br. J. Nutr.* **40**, 377–386.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES