IV Nutritional Chemistry of Inorganic Trace Constituents in the Diet

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Although improvements in techniques have greatly increased the precision with which the inorganic trace constituents of a diet can be determined,[1] nutritional studies have clearly shown that analytical values for individual elements are not sufficient to allow one to predict whether an animal would be able to obtain adequate amounts of these elements from the diet.[2,3] Similarly, it is often difficult to predict the deleterious effects of toxic trace elements merely by determining their dietary concentrations.[4] These difficulties are now known to result from interactions between the elements, from differences in their chemical forms, and from influences exerted by other dietary constituents.

A well known example of the influence of the chemical form of an element is the difference between the biological potency of Co as an inorganic salt and as a component of vitamin B_{12}. Only as the latter compound will Co fulfil its essential role for most animals, and therefore estimates of total dietary Co intake are insufficient. The only exception to this is for ruminant animals where the micro-organisms of the rumen are able to convert Co to vitamin B_{12} and supply the animal's needs. Problems arise even in this situation, however, because a variable proportion of the dietary Co is converted into analogues of vitamin B_{12} which are inactive in the host animal.[5] A somewhat similar situation seems to be emerging with regard to Cr nutrition of most animals including man. Current research suggests that Cr is required in the form of a specific organic complex, glucose tolerance factor, for it to be fully active in potentiating the functions of insulin.[6] The precise structure of this complex is unknown[7] but appears to represent a variable fraction of the total Cr content of a diet. Analyses of the latter therefore

[1] C. J. Pickford, *Chem. Soc. Rev.*, 1981, **2**, 245.
[2] I. Bremner and N. T. Davies, *Rep. Rowett Inst.*, 1973, **29**, 126.
[3] N. T. Davies, *Proc. Nutr. Soc.*, 1974, **33**, 293.
[4] N. T. Davies, *Proc. Nutr. Soc.*, 1979, **38**, 121.
[5] E. J. Underwood, 'Trace Elements in Human and Animal Nutrition', 4th edition, Academic Press, London, 1977, p. 146.
[6] R. A. Anderson, M. M. Polansky, J. H. Brantner, and E. E. Roginsk in 'Trace Element Metabolism in Animals', ed. M. Kirchgessner, Arbeitskreis für Tierernährungsforschung, Weihenstephan, West Germany, 1978, p. 269.
[7] W. Mertz, R. A. Anderson, W. R. Wolf, and E. E. Roginski in 'Trace Element Metabolism in Animals', ed. M. Kirchgessner, Arbeitskreis für Tierernährungsforschung, Weihenstephan, West Germany, 1978, p. 272.

provide inadequate information to assess the adequacy of the diet with regard to Cr.[8,9,10]

The above examples of a nutritional requirement for a specific pre-formed complex appear to be exceptional. However, most of the trace metals tend to form complexes with organic constituents of the diet, and these frequently render the metals insoluble and not available for absorption. Entry of these complexes into the acid medium of the stomach often results in dissociation of the dietary complexes. However, subsequent neutralization of the digesta during passage down the small intestine results in recombination of the metals with dietary ingredients and digestive juices leading to the formation of a whole new range of complexes.[11,12] In sheep, for example, the low pH of the stomach resulted in the expected release of Zn and Mn into the soluble fraction of the digesta, but the solubility of Cu actually decreased.[12] It is possible that this resulted from the formation of insoluble cupric sulphide when both the cupric and sulphide ions were liberated from previously soluble complexes by the acid of the abomasum. In man a mucopolysaccharide secreted by the stomach binds Fe liberated from dietary complexes by the fall in pH and appears to retain the Fe in a soluble and available form during subsequent neutralization of the digesta.[13]

The formation of a complex salt during neutralization of the digesta also appears to underlie the profound effects of dietary phytic acid [*myo*-inositol 1,2,3,4,5,6-hexakis(dihydrogen phosphate)] on the availability of Zn, Cu, and Mn from diets.[14] Diets based on maize and soya-bean products, which are rich in phytic acid, have in the past caused parakeratosis, symptomatic of Zn deficiency, in pigs.[15] Delayed sexual maturation in man in the Middle East has also been associated with Zn deficiency caused by a staple diet of unleavened bread rich in phytic acid.[16,17] At neutral to alkaline pH in conditions similar to those in the gut, phytic acid forms insoluble salts containing predominantly Ca but also Zn, Cu, and Mn.[18] These salts appear to pass relatively unchanged through the absorptive regions of the gut and thus render the associated trace metals unavailable. For any given Ca concentration the extent of precipitation of Zn depends on its molar ratio to the phytic acid, and the ratio of phytate to Zn has also been shown to control the availability to rats of Zn from diets with relatively

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[9] R. A. Anderson, J. H. Brantner, and M. M. Polansky, *J. Agric. Food Chem.*, 1978, **26**, 1219.

[10] C. T. Gurson, *Adv. Nutr. Res.*, 1977, **1**, 23.

[11] I. Bremner and A. H. Knight, *Br. J. Nutr.*, 1970, **24**, 279.

[12] I. Bremner, *Br. J. Nutr.*, 1970, **24**, 760.

[13] A. Jacobs and P. M. Miles, *Br. Med. J.*, 1969, **4**, 778.

[14] N. T. Davies and R. Nightingale, *Br. J. Nutr.*, 1975, **34**, 243.

[15] P. K. Lewis, W. G. Hoekstra, R. H. Grummer, and P. H. Phillips, *J. Anim. Sci.*, 1956, **15**, 741.

[16] J. A. Halsted, H. A. Ronaghy, P. Abadi, M. Haghshenass, G. H. Amirhakemi, R. M. Barakat, and J. G. Reinhold, *Am. J. Med.*, 1972, **53**, 277.

[17] J. G. Reinhold, A. Parsa, N. Karimian, J. W. Hammuk, and F. Ismail-Beigi, *J. Nutr.*, 1974, **104**, 976.

[18] N. T. Davies and S. E. Olpin, *Br. J. Nutr.*, 1979, **41**, 590.

Table 1 *Effect of increasing concentrations of phytic acid on the solubility of* Zn *at pH* 6.3 *in vitro and on the growth of rats[a]*

Phytate: Zn ratio	Zn remaining[b] soluble at pH 6.3 in vitro/%	Average daily weight gain[c]	
		6 g Ca kg^{-1}/g	12 g Ca kg^{-1}/g
0	—	5.76 ± 0.27[d]	5.78 ± 0.48[d]
10	2.08	5.01 ± 0.15	6.34 ± 0.26
15	1.85	5.81 ± 0.37	5.60 ± 0.23
20	1.63	5.13 ± 0.16	4.11 ± 0.20
30	1.40	4.94 ± 0.18	3.16 ± 0.23

[a] Data from ref. 18. [b] The solutions contained 2.4 g Ca l^{-1} and 7.2 mg Zn l^{-1}. [c] The diets contained 6 or 12 g Ca kg^{-1} and 18.5 mg Zn kg^{-1}. [d] Values are means ± S.E.

high Ca contents.[18,19] However, in assessing the availability of Zn from diets containing phytic acid, the Ca content of the ration is at least equally important.[20] Thus parakeratosis in pigs was severely aggravated by an increase in the Ca content of their ration from 8 g kg^{-1} to 12 g kg^{-1} [15] and, in rats, phytate:Zn ratios that severely impaired growth on diets containing 12 g Ca kg^{-1} failed to influence it when the dietary Ca concentration was only 6 g kg^{-1}.[18] Furthermore a recent study has shown that even moderately high phytate:Zn ratios can be tolerated when the total Zn present is substantially above the minimum required in the absence of phytate, and the Ca intake is not excessive.[20] These findings together suggest that equilibria are established within the gut between soluble Ca ions and phytic acid which result in the precipitation of a portion of the latter as the insoluble Ca salt. The availability of the trace metals present depends on the extent to which these divalent metals substitute for Ca in the precipitated phytates.

Interest in the potential influence of dietary phytates on the trace-metal status of man has increased recently with the trend towards substitution of meat by products based on processed soya-bean protein. Until recently meat has been a major dietary source of readily available trace metals, whereas the highly processed, textured vegetable proteins tend to have relatively low concentrations of Zn and are rich in phytic acid.[21] Furthermore animal studies of phytic acid metabolism have shown that the enzyme responsible for partial degradation of phytic acid in the gut, phytase, appears to be Zn dependent.[22] Any reduction in availability of Zn caused by phytic acid in the diet may therefore reduce the activity of phytase in the gut, increase the proportion of the dietary phytate, which remains undegraded, and thus aggravate the reduction in Zn availability. Nevertheless the importance of phytic acid in determining man's trace-element status may be less than animal studies have suggested because of the relatively

[19] D. Oberleas, *Proc. West. Hemisph. Nutr. Congr.*, 1975, **IV**, 156.
[20] E. R. Morris and R. Ellis, *J. Nutr.*, 1980, **110**, 1037.
[21] N. T. Davies and H. Reid, *Br. J. Nutr.*, 1979, **41**, 579.
[22] N. T. Davies and A. Flett, *Br. J. Nutr.*, 1978, **39**, 307.

low Ca intakes of man[23] and the general diversity of ingredients within single meals. Furthermore whole-meal bread, which can be a major source of phytic acid in human diets, has been shown to have a higher Zn content than white bread.[23a] Therefore, although the fractional absorption of dietary Zn was less from diets containing whole-meal bread, the amount of Zn absorbed was actually greater than when white bread was eaten.

In addition to phytic acid the fibre components of plant products have been implicated in binding the essential trace elements. Studies with rats showed a 32 % decrease in the fractional absorption of Zn when 6 % cellulose was added to a semi-synthetic diet,[24] and 14.2 g cellulose/d added to the diet of a group of adolescent boys increased by over 30 % the faecal excretion of Zn and Cu.[25] Furthermore, when diets supplying close to the recommended daily allowances of Zn and Cu were offered to men, they remained in positive balance for these elements if the diets were low in fibre but showed a net loss of Zn and Cu when the diets were rich in fibre from fruit and vegetables.[26] Similarly supplementation of human diets with hemicellulose markedly reduced the fractional retention of Zn.[27] Bacterial cell walls have also been found to bind trace elements[28] and may be partially responsible for an observed increase in trace-element requirements in conventionally reared animals relative to their germ-free equivalents.[29] In the walls of *Bacillus subtilis* the metals were bound to carboxyl groups,[30] but a wider investigation of the nature and degree of binding to bacterial cell walls in general will be needed before their potential influence on trace-metal availability can be assessed.

Although the formation of complexes between trace inorganic constituents and the organic components of diets frequently reduces the availability of the inorganic components, these complexes can also enhance availability. Mention has already been made of the situations where these complexes remain intact during absorption and function as vitamins within the animal, but other complexes appear to be important only during the actual processes of absorption. Although most biological forms of Fe appear to be equally available to animals, Fe present in the diet as part of the haem molecule is preferentially absorbed.[31] Typical values for the fractional absorption of Fe have been reported as 20—25 % of haem Fe and only 3—8 % of non-haem Fe. Calculations of the adequacy of Fe

[23] 'Household Food Consumption', Ministry of Agriculture and Fisheries, London, 1980.
[23a] B. Sandstrom, B. Arvidsson, A. Cederblad, and E. Bjorn-Rasmussen, *Am. J. Clin. Nutr.*, 1980, 33, 739.
[24] W. M. Becker and W. G. Hoekstra in 'Intestinal Absorption of Metal Ions, Trace Elements and Radionuclides', ed. S. C. Skoryna and D. Waldron-Edward, Pergamon Press, Oxford and New York, 1971, p. 229.
[25] L. M. Drews, C. Kies, and H. M. Fox, *Am. J. Clin. Nutr.*, 1979, 32, 1893.
[26] J. L. Kelsay, R. A. Jacob, and E. S. Prather, *Am. J. Clin. Nutr.*, 1979, 32, 2307.
[27] C. Kies, H. M. Fox, and D. Beshgetor, *Cereal Chem.*, 1979, 56, 133.
[28] C. F. Mills, *Soil Sci.*, 1958, 85, 100.
[29] J. C. Smith, E. G. McDaniel, L. D. McBean, F. S. Doft, and J. A. Halsted, *J. Nutr.*, 1972, 102, 711.
[30] R. J. Doyle, T. H. Matthews, and U. N. Streips, *J. Bacteriol.*, 1980, 143, 471.
[31] E. R. Monsen, L. Hallberg, M. Layrisse, D. M. Hegsted, J. D. Cook, W. Mertz, and C. A. Finch, *Am. J. Clin. Nutr.*, 1978, 31, 134.

intake of man based on dietary analyses must therefore take into account the proportion of the element present as haem. However, Fe absorbed in this form appears to be liberated from the porphyrin moiety within the mucosa and thereafter enters the same metabolic pool in plasma, as does the dietary non-haem Fe.[32]

Absorption of non-haem Fe occurs preferentially in the ferrous form and is markedly enhanced by ascorbic acid in the diet.[33] The latter appears to act both as a reducing agent and as a chelator of Fe within the gut. A wide range of other low molecular weight substances capable of forming chelates with Fe also facilitate absorption of non-haem Fe. Among these is citric acid, which has also been suggested to be involved in the absorption of Zn from milk.[5,34]

Milk diets have long been recognized to result in relatively high fractional absorptions of many essential and toxic trace elements.[35,36] In many instances, however, the animals receiving these diets have been young, and the effects of milk on absorption have been confounded by those of age, relatively high fractional absorptions of the trace metals being characteristic of the neonates of many species.[35,36] However, the studies by Kostial[36] have shown that, even with

Table 2 *Influence of age and milk diet on trace-metal absorption*[a]

	Absorption/% *of oral dose*		
Element	*Sucklings*	*Weaned animals*	
		Milk diet	*Standard diet*
Pb	52	23	0.4
Cd	26	7	0.5
Mn	40	6	0.05

[a] Data from ref. 36.

previously weaned rats, a return to a liquid milk diet increased the retention of oral doses of Pb, Cd, and Mn. This effect is largely dependent on milk being the sole nutrient. When fed with solid diets its effects have been much less dramatic, and in some instances milk has even reduced the uptake of trace metals.[37] Furthermore, the availability of trace elements from milk may vary with the species of origin of the milk. Thus there is a rare inherited disease of man, acro-dermatitis enteropathica, associated with an inability to absorb adequate amounts of Zn from normal diets. Generally the sufferers are able to obtain adequate

[31] L. R. Weintraub, M. B. Weinstein, H.-J. Huser, and S. Rafal, *J. Clin. Invest.*, 1968, **47**, 531.
[33] J. D. Cook and E. R. Monsen, *Am. J. Clin. Nutr.*, 1977, **30**, 235.
[34] B. Lonnerdal, A. G. Stanislowski, and L. S. Hurley, *J. Inorg. Biochem.*, 1980, **12**, 71.
[35] C. F. Mills and N. T. Davies in 'Development of Mammalian Absorptive Processes', C.I.B.A. Symposium No. 70, Excerpta Medica, Amsterdam and Oxford, 1979, p. 247.
[36] K. Kostial, D. Kello, S. Jugo, I. Rabar, and T. Maljkovic, *Environ. Health Perspect.*, 1978, **25**, 81.
[37] J. Quarterman and E. Morrison, *Proc. Nutr. Soc.*, in press.

amounts of Zn while receiving human milk but develop the symptoms of the disease if transferred to cow milk or solid diets.[38] This does not result from simple differences in Zn content since human milk generally contains a lower concentration of Zn than bovine milk and most normal diets. Early comparisons of human and bovine milk showed that despite the former's lower total Zn content it had a higher concentration of Zn associated with the low molecular weight fraction. This led to the hypothesis that the high availability of Zn from human milk was caused by the presence of a low molecular weight chelator which facilitated absorption.[39] One group has suggested that citric acid is the critical compound,[34] yet bovine milk contains higher concentrations of citric acid than human milk,[40] and rat milk, which is essentially devoid of citric acid, gave the highest availability of Zn when this was tested with rats.[41] Picolinic acid, a metabolite of tryptophan, has also been suggested,[42] but Cousins[40] considered that none of these compounds has sufficiently high binding constants for Zn to be significantly better chelators of it than many of the amino-acids present. His view was that the higher protein concentration of bovine milk results in most of the Zn being bound to high molecular weight components and that the Zn in low molecular weight form in human milk is not bound to any unique component. However, overshadowing the above controversy is the lack of convincing evidence that the differences in availability actually depend on the nature and proportions of the Zn in low molecular weight complexes. Furthermore a recent study has shown that lactoferrin, a protein present in much higher concentration in human milk than in bovine, also binds Zn.[43] The presence of species-specific proteins which facilitate trace-metal absorption from milk cannot be ruled out. The influence of milk on trace-metal absorption clearly needs further investigation. It may even be found that much of the effect of milk on absorption is caused by its high digestibility resulting in relatively little faecal residues and therefore little competition for trace metals between non-digested solids and the animals' transport systems.

Trace-metal availability is influenced by compounds secreted into the gut as well as those derived from the diet, and the influence of gastric mucopolysaccharides on Fe absorption has already been mentioned. The absorption of Pb depends markedly on bile components and was reduced ten-fold when bile flow was diverted from the gut of rats.[44] There have also been reports of low molecular weight ligands able to bind trace metals in the lumen of the gut and in the cytosol of mucosal cells.[45,46] These ligands have been variously characterized, but present evidence suggests that they may be artefacts derived from a metal binding pro-

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[38] V. M. de Kaloustian, S. S. Musallan, S. A. Sanjad, A. Murib, W. D. Hammad, and Z. H. Idriss, *Am. J. Dis. Child.*, 1976, **130**, 421.

[39] L. S. Hurley, J. R. Duncan, M. V. Sloan, and C. D. Eckhert, *Proc. Nat. Acad. Sci. U.S.A.*, 1977, **74**, 3547.

[40] R. J. Cousins and K. T. Smith, *Am. J. Clin. Nutr.*, 1980, **33**, 1083.

[41] P. E. Johnson and G. W. Evans, *Am. J. Clin. Nutr.*, 1978, **31**, 416.

[42] G. W. Evans and P. E. Johnson, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1979, **38**, 703.

[43] E. W. Ainscough, A. W. Brodie, and J. E. Plowman, *Am. J. Clin. Nutr.*, 1980, **33**, 1314.

[44] J. Quarterman, J. N. Morrison, and W. R. Humphries, *Proc. Nutr. Soc.*, 1977, **36**, 103A.

[45] J. R. Duncan and L. S. Hurley, *Am. J. Physiol.*, 1978, **235**, E556.

[46] B. R. Schricker and R. M. Forbes, *Nutr. Rep. Int.*, 1978, **18**, 159.

tein, metallothionein, which has been partially degraded by digestive enzymes during the preparation of extracts.[47,48]

As well as containing naturally occurring binding agents diets have frequently been supplemented with chelators or chelated trace metals with the intention either of increasing the availability of dietary trace minerals or of supplying available forms of the trace metals.[49-51] The addition of EDTA to phytate-rich diets was found to increase the availability of Zn to turkeys,[52] and in subsequent studies with both turkeys and chicks several chelating agents were investigated[50,53] (see the Figure). The diets fed to the turkeys contained phytate-rich
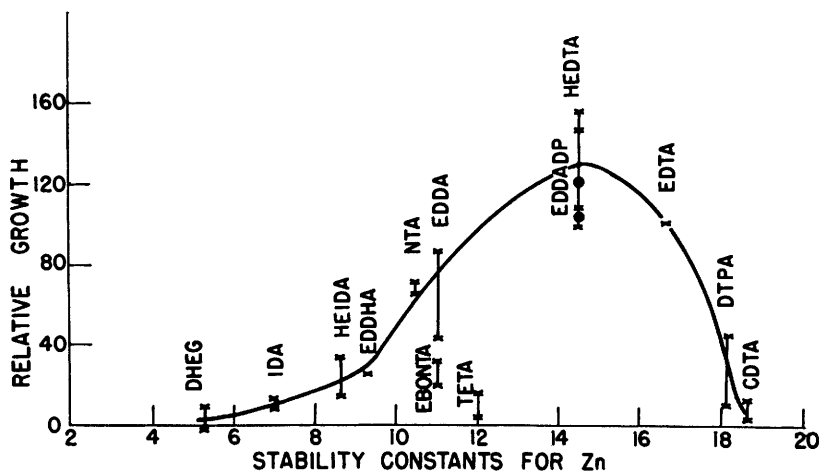

Figure *Relation of stability constant for* Zn *of several chelating agents to their growth-promoting effects in turkey poults when added to a* Zn-*deficient diet*
(Reproduced with permission from *J. Nutr.*, 1964, **82**, 249)

soya-bean protein and only 26 mg Zn kg^{-1}. They were inadequate in available Zn as shown by a major response in growth to Zn supplementation. Adding to the basal diet chelators with log stability constants for Zn from 13 to 17 resulted in growth responses similar to those obtained with Zn. Chelators of lower or higher affinity for Zn produced lesser growth responses. It was suggested that below the critical range of affinities the chelators were unable to sequester Zn from the dietary ingredients while above the range Zn removed from the diet by the chelator was so firmly bound that it was unavailable to the animal and was

[47] R. J. Cousins, *Nutr. Rev.*, 1979, **37**, 97.
[48] R. J. Cousins, K. T. Smith, M. L. Failla, and L. A. Markowitz, *Life Sci.*, 1978, **23**, 1819.
[49] C. L. Keen, B. Lonnerdal, M. V. Sloan, and L. S. Hurley, *J. Nutr.*, 1980, **110**, 897.
[50] F. H. Nielsen, R. L. Sunde, and W. G. Hoekstra, *J. Nutr.*, 1966, **89**, 35.
[51] N. W. Solomons, R. A. Jacob, O. Pineda, and F. E. Viteri, *J. Nutr.*, 1979, **109**, 1519.
[52] F. H. Kratzer, J. B. Allred, P. N. Davis, B. J. Marshall, and P. Vohra, *J. Nutr.*, 1959, **68**, 313.
[53] P. Vohra and F. H. Kratzer, *J. Nutr.*, 1964, **82**, 249.

excreted along with the chelator. Within the critical range Zn removed from dietary ingredients by the added chelator was still sufficiently labile to be taken up by the animals' tissues and be used in its essential metabolic roles.

The organic components of a diet can also influence the utilization of trace inorganic constituents even after they have been absorbed. Thus it has been known for many years that a range of substances, the goitrogens, will impair iodine metabolism. Research has shown that they can be divided into two groups according to their mode of action. Those of the cyanogenetic type inhibit uptake of I by the thyroid gland and are thought to act by giving rise to thiocyanate or isothiocyanate ions which are of similar ionic size to the iodide ion and compete with it for the iodide transport system of the gland.[54] The other main group of goitrogens, the thio-oxazolidones, prevent iodination of tyrosine residues within the gland, presumably by inhibiting iodide peroxidase, and their effects are not generally reversible by increasing the I content of the diet.

A wide range of mutual interactions between the trace metals also influence their availability. Hill and Matrone[55] first advanced the hypothesis that ions having similar electronic structure might compete with each other in metabolic pathways. High concentrations of Zn in the diets of chicks reduced growth rate and survival, and these effects were found to be reversible on increasing the Cu content of the diet.[55] Similar interactions have also been observed when Cd was added to the diet.[56] Subsequent studies have confirmed many of the postulates of Hill and Matrone and have shown that interactions can occur between Cu, Zn, and Cd and between Pb and Ca and Mo and W. In many instances the underlying mechanisms have still to be clarified, but the induction of Cu deficiency by high dietary concentrations of Zn or Cd is better understood. This appears to originate from the induction by Zn or Cd of a protein, metallothionein, in the gut mucosa.[57] Cysteine residues account for almost 30% of the amino-acids in metallothionein, which acts as storage protein for several types of divalent cation. Even when induced by one cation such as Zn^{2+} or Cd^{2+} the protein is still capable of binding other metal ions such as Cu^{2+} whose passage through the mucosal cells towards the bloodstream is thus interrupted. Since the normal functioning of the mucosal epithelium results in sloughing of the cells as they reach the tip of the villus, any metals still bound to metallothionein are returned to the lumen of the gut. High dietary concentrations of Zn and Cd therefore interfere with the absorption of Cu. Another likely source of interaction results from competition of analogous ions for transport systems. It seems likely that such a mechanism underlies the antagonistic effects of the SO_4^{2-}, MoO_4^{2-}, and WO_4^{2-} ions.[58]

Interactions between trace metals occur not only during absorption but also within the body. Thus high dietary concentrations of Ca reduced the fraction of

[54] N. T. Davies, *Proc. Nutr. Soc.*, 1979, **38**, 121.
[55] C. H. Hill and G. Matrone, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1970, **29**, 1474.
[56] I. Bremner and J. K. Campbell, *Environ. Health Perspect.*, 1978, **25**, 125.
[57] A. C. Hall, B. W. Young, and I. Bremner, *J. Inorg. Biochem.*, 1979, **11**, 57.
[58] C. J. Cardin and J. Mason, *Biochim. Biophys. Acta*, 1976, **455**, 937.

Table 3 *Effect of dietary Zn concentration on the absorption of an oral dose of $^{64}Cu^a$*

Dietary[b] Zn concentration/mg kg^{-1}	Fraction of dose of ^{64}Cu absorbed/%	^{64}Cu on metallo-thionein in mucosal cpm mg^{-1}	Concentration of Zn on metallo-thionein in mucosal µg g^{-1}
30	43	10	0.9
150	39	11	1.6
450	43	17	3.4
900	27	63	24.4

[a] Data from ref. 57. [b] All diets contained 3 mg Cu kg^{-1}.

Pb absorbed by rats but also reduced the rate of release of Pb from the body once Pb was removed from the diet.[59,60] The interaction during absorption probably resulted from competition for transport proteins since low dietary Ca intakes stimulated Pb absorption and vitamin D affected Pb uptake in a manner similar to its effect on Ca transport.[61] However, the influence of Ca on the retention of Pb, which had been previously absorbed, was less readily explicable since both low and high intakes of Ca increased the retention of Pb relative to diets containing normal amounts of Ca.[59,60]

Once trace inorganic constituents have been absorbed they may be temporarily stored in organs such as the liver prior to being utilized for their essential functions or being excreted. During each of these processes their fate is often influenced by interactions with other essential or toxic elements. For example, high dietary Cd concentrations tend to increase liver Zn storage as a result of induction of metallothionein synthesis in the liver,[62] and the toxic effects of Cd on the testis can be reduced by increasing the Zn intake of the animal.[63]

These and many other aspects of research into the metabolism of trace elements merit closer examination by chemists from all branches of the science. The history of attempts to understand the influence of milk on trace-metal absorption can be used to illustrate this. In order to identify factors which influence absorption, milk must be separated into its components. The first approach has been to fractionate it on the basis of particle size, but attempts to use gel exclusion chromatography or molecular filters have been hindered by the inherent ion-exchange properties of these materials. Although slight and often stated to be insignificant, metal binding by these systems is often excessive for satisfactory separations of the trace metals present in biological samples. Chemical modifications devised to reduce these binding affinities would be invaluable.

When separated on the basis of particle size, milk contains complex micelles

[59] J. Quarterman and J. N. Morrison, *Br. J. Nutr.*, 1975, **34**, 351.
[60] J. Quarterman, J. N. Morrison, and W. R. Humphries, *Environ. Res.*, 1978, **17**, 60.
[61] C. M. Smith, H. F. DeLuca, Y. Tanaka, and K. R. Mahaffey, *J. Nutr.*, 1978, **108**, 843.
[62] M. D. Stonard and M. Webb, *Chem.-Biol. Interact.*, 1976, **15**, 349.
[63] J. Parizek, *J. Endocrinol.*, 1957, **15**, 56.

accounting for much of its protein and lipid. A substantial proportion of the metals present is also in these micelles.[64] A better understanding of their structure and physical chemistry and of the processes governing partition of metals between aqueous, lipid, and insoluble phases would aid assessment of their importance in modifying metal absorption.

A recurrent theme in studies of the trace metals has been their binding to the other components present in biological systems, for example the low molecular weight constituents of milk. Attempts to assess the relevance of this binding have been hindered by the lack of mathematical methods for assessing the complex interactions of binding species. Even where such methods are being developed their usefulness has often been limited by lack of suitable data. There is still a great need for estimates of metal-binding affinities which have been determined under physiologically relevant conditions of pH, ionic strength, and temperature. Methods are also needed for including in simulations of binding equilibria the high molecular weight, multi-ligand compounds such as proteins, which play such an important role in biological situations.

Finally, although analytical methods for the trace elements have improved immensely, there is still a need for ultra-micro methods of analysis. At present, metal concentrations generally have to be determined by methods which tend to average out variations in concentration within a tissue or cell. In many instances these structures must be disrupted and fractions pooled to obtain adequate quantities for analysis. The consequent disruption of natural compartmental barriers provides ample opportunity for redistribution of the metals *in vitro* and causes uncertainty as to the relevance of the results to the situation *in vivo*. Methods such as electron-probe microscopy and laser-probe atomic absorption or fluorescence applied to frozen-dried tissue preparations would, if developed to a sufficient degree of sensitivity, greatly aid our understanding of the biological location and role within animals of the trace inorganic constituents of diets.

[64] J. E. Piletz and R. E. Ganschow, *Am. J. Clin. Nutr.*, 1979, **32**, 275.