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DIETARY INTERACTIONS INVOLVING THE TRACE ELEMENTS

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INTRODUCTION

The effect of variations in the form and content of inorganic constituents of diets on the metabolic fates of both essential and toxic trace elements is often substantial. Attempts to define the minimal trace element requirements of man and animals are commonly hindered by not taking into account the influence of such variables on the efficiency of absorption of essential elements. Superficial understanding of how such interactions arise also limits our ability to anticipate the development of deficiency or to predict the influence of diet upon susceptibility to trace element toxicity.

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Present understanding of the nutritional and toxicological significance of such interrelationships has been gained largely from a series of circumstantial observations. One exception is the attempt to provide a rational explanation of mutual antagonistic effects of structurally related inorganic elements or ions that are believed to share common binding sites on receptors involved in their absorption, transport, and tissue distribution. This concept (59) proves particularly fruitful in the design of experiments that have now revealed the wide range of interactions that may be nutritionally or toxicologically relevant. Such interactions are summarized elsewhere (10, 80). A second, if more restricted, area of rapid and systematic growth developed from intensive studies of the roles of metallothionein-like, low-molecular-weight, sulfur-rich proteins as determinants of the kinetics of tissue distribution, utilization, or toxicity of the elements zinc, cadmium, copper, and mercury (7, 63). Once it became established beyond doubt that the metallothioneins had roles not merely restricted to the sequestration of potentially toxic tissue concentrations of cadmium but also acted as modulators of the tissue flux of zinc and copper (9), with both competing for its metal binding sites, a rational approach to the study of at least one important group of metal-metal interactions became feasible.

Such developments have materially enhanced the sensitivity of studies of trace metal–related interactions. This is evident, for example, in recent experiments indicating that "nutritionally realistic" increases in zinc intake depress cadmium retention by some tissues (56, 62) and, as discussed below, may influence iron and copper utilization (62). Until recently, much of the data describing the effects of these and other interactions was derived from experiments imposing very wide variations in dietary composition, especially with respect to the concentrations of potentially competitive inorganic elements. While often appropriate for exploring aspects of the biochemistry of such interactions, the relevance of such data to circumstances other than those arising from gross, dietary contamination or "environmental" anomalies is often in doubt. This point is also emphasized in two recent reviews (28, 38). Thus, the present chapter is restricted to interactions whose nutritional relevance is strongly suspected but whose quantitative effects are difficult to predict and whose mechanisms are sometimes insufficiently well understood to permit confident extrapolation between species in the context of human and animal nutrition. Even in such situations there are often reasonable grounds for suspecting that the events modifying trace element utilization or metabolic responses to deficiency or toxicity occur (*a*) prior to absorption, (*b*) at some stage during the absorptive process, or (*c*) systemically, during the physiological utilization of elements within body tissues. The examples considered are categorized accordingly.

VARIABLES INFLUENCING INTESTINAL SOLUBILITY OF TRACE ELEMENTS

Dietary Phytate, Calcium, and Trace Metals

The role of dietary phytate as an antagonist of zinc absorption has been known for 25 years (14, 73, 94, 96). Despite this, substantial controversy still exists as to the significance of phytate as a determinant of the availability of dietary zinc to most species other than rats and chicks.

Early in vitro studies of reactions between soluble phytate, zinc, and calcium to yield the zinc/calcium/phytate complex, [Zn Ca phytate], at the pH of the distal duodenum suggested that the inhibitory effect of phytate on the utilization of zinc is explicable on the basis of intraluminal reactions depressing zinc solubility (11). This convincing argument has been widely accepted but often without considering the restricted physiological relevance of the simplified aqueous systems within which such evidence was first obtained.

Preeminent among the variables presenting difficulty in attempts to predict zinc availability from consideration of dietary phytate content must be the form and stability toward hydrolysis of ingested phytate and, secondly, the intraluminal presence of other ligands that, by associating with zinc, reduce the concentration of Zn^{2+} ions free to participate in reactions leading to formation of [Zn Ca phytate]. The importance of such variables is frequently obscured in studies of the phytate/zinc interaction with laboratory rats offered semisynthetic diets formulated to meet recommended nutrient allowances for all constituents other than "available" zinc. Data obtained under such conditions (26, 88) indicate unequivocally that dietary phytate:Zn (molar) ratios exceeding 25:1 sufficiently inhibit zinc utilization to restrict growth, provided dietary calcium is maintained at or above the currently accepted RDA for the rat of 5 g Ca/kg diet (91). However, attempts to predict the availability of zinc in human diets by use of the phytate/zinc ratio technique have not been uniformly successful (19, 38). The reasons for this are now becoming clearer. The fact that calcium contents of human diets are markedly lower than those of typical rat bioassay diets clearly contributes to this difficulty. Thus recent quantitative studies of the synergistic effects of dietary calcium upon the phytate/zinc antagonism show that variations in Ca content between 3 and 6 g/kg have a major influence on the availability of zinc when phytate is present (16, 88). The dominant role of calcium in determining zinc availability in phytate-rich diets is now becoming understandable from evidence of its role as a determinant of phytate solubility (135) and thus of the yield of the [Zn Ca phytate] complex (134). Furthermore, the rate of phytate hydrolysis by phytases of the intestinal mucosa appears to be inversely proportional to the intraluminal concentration of calcium (90, 137). Reappraisals of published data from experiments with rats in

which the phytate/zinc antagonism was studied using diets ranging in Ca content from 3 to 17.5 g/kg indicate a substantially greater predictive value of equations describing zinc availability when the equations incorporate a term accounting for the synergistic effect of calcium. Thus in a recent study (85) the rate of weight gain of rats (ΔW; g/day) was closely related to the (molar) concentration of dietary Ca, Zn, and phytate by the expression:

$$\Delta W = 6.213 - 0.331x \qquad (r=0.877;\ t=11.77),$$

where x = [Ca][phytate]/[Zn] moles/kg diet; and the critical threshold ratio of [Ca phytate]/[Zn] above which available zinc supply was inadequate to maintain growth was approximately 3.5. This relationship is clearly of greater value for predicting zinc availability from the diets of rodents; its predictive value for human diets cannot yet be assessed because of the startling infrequency with which concentrations of all relevant variables are reported. However, preliminary estimates (23) derived from the few recent studies with human subjects in which such data are presented (21, 42) or can readily be calculated (92) suggest that a ratio exceeding 0.4 may significantly reduce the efficiency of zinc absorption, or if exceeding 3, produce evidence of depletion of tissue zinc.

While the predictive value of the [Ca][phytate]/[Zn] ratio may well prove to be superior to that of the phytate/zinc ratio, it must also be anticipated that either will have important limitations when applied to dietary regimes differing markedly with respect to amino acid pattern and protein content. This belief arises from the results of balance studies with human subjects (110) and zinc availability studies with rats (24), which indicate that the phytate/zinc antagonism is ameliorated by increases in dietary protein. It is strengthened by evidence that the free amino acids, histidine, cysteine, and methionine readily desorb zinc from the particulate, insoluble [Zn Ca phytate] complex (136). Other reports describe the extent to which ligands normally present in the soluble phase of intestinal lumen contents of rats are capable of dissolving a range of metals (M) from insoluble [M Ca phytate] complexes. The yield of soluble copper or zinc from such complexes was directly proportional to the amino-N content of the intestinal soluble phase, and the effectiveness of release of a range of metals from their complexes with phytate was typically Cu>Cd>Mn>Zn>Pb (136). Such data begin to provide a rational explanation of a number of phenomena associated with the consumption of phytate-rich diets. Thus they indicate why absorption of zinc is more strongly inhibited than that of copper (20, 25, 97) and they support evidence from recent studies with rats indicating that, contingent upon their calcium content, such diets strongly inhibit the absorption of dietary lead (108, 109, 132). The possible value of high phytate diets for restricting lead accumulation by workers exposed to lead in industrial environments is being investigated.

In the face of physicochemical data indicating that the insolubility and biological stability of phytate-containing metal complexes are determined substantially by their alkaline-earth component (19, 134), and given evidence that magnesium deficiency can also be induced by a high phytate diet (107, 112), it is realistic to anticipate that future attempts to predict the inhibitory effects of dietary phytate on trace metal absorption must include consideration of both dietary calcium and magnesium content. Although [Mg phytate] complexes are already known to have a strong affinity for zinc (134), the synergistic effects of dietary magnesium upon the phytate/zinc antagonism have not yet been fully investigated. However, one recent study (39) suggests that Ca- and Mg-phytates may be equally effective inhibitors of zinc absorption.

Provided that the complexity of equilibria governing the antagonistic effect of phytate upon the availability of several metals is recognized in the design and in published accounts of experimental protocols, there are grounds for optimism that controversy as to the nutritional significance of phytate may soon diminish. However, it is also suspected that the biopotency of phytate is affected not only by food-processing techniques that modify the solubility or susceptibility to hydrolysis of the indigenous phytate/protein complexes present in many foods (19, 31, 38) but also by developmental changes in intestinal phytate activity (21, 93, 133, 134), some of which are associated with the transition from liquid to solid diets.

Absorption and Metabolism of Copper

The efficacy with which copper is absorbed from the intestine is influenced both by reactions affecting its intraluminal solubility and by competitive interactions modifying its fate during transport through the mucosa. Direct or indirect evidence of the nutritional relevance of the "mucosal block" to copper absorption induced by high dietary concentrations of zinc or of cadmium was considered extensively in several recent reviews (e.g. 70, 81) and is not reconsidered here. Instead, the following sections emphasize aspects of interactions that influence the intralumenal solubility of copper and, in the instance of those involving sulfur, molybdenum, and iron, not only inhibit copper absorption but are also involved in the etiology of copper deficiency.

POLYURONIC ACID DERIVATIVES AND PHYTATE Many naturally occurring organic or inorganic ligands present in the diet or gastrointestinal secretions exhibit an affinity for copper and react therewith to yield products that are sparingly soluble in aqueous systems. The most common dietary constituents exhibiting such properties are the "fiber" and "phytate" fractions. However, despite evidence that, for example, the polygalacturonic residues of pectins readily bind copper at neutral pH (98) to form insoluble products, the metal

complexes formed with the structurally related ligands of relatively indigestible polysaccharides such as alginates, pectates, and hemicelluloses appear to be readily degraded. Thus such constituents have little or no significant effect upon copper utilization (for further details see 38, 57). Experimentally, there is no doubt that phytate reacts with Cu to form stable insoluble complexes (134) and, if added as a soluble phytate to the diet, can reduce copper absorption by rats (25) or provoke clinical signs of copper deficiency in chicks (27). Again, however, there are no indications that the concentrations of phytate encountered in typical human diets sufficiently inhibit the utilization of dietary copper to give rise to concern (65, 126).

DIETARY SULFUR AND MOLYBDENUM AS COPPER ANTAGONISTS In marked contrast to the above, some metabolites of dietary sources of sulfur significantly inhibit copper utilization, particularly in the nutrition of ruminants. The fractions of ingested sulfate, methionine, and cyst(e)ine metabolized via sulfide (S^{2-}) or hydrosulfide (HS^-) ions by rumen microorganisms are highly variable (5, 6) for reasons that are not yet defined adequately. Nevertheless, it is clear, firstly, that the flow of soluble copper from rumen to abomasum and the small intestine is inversely proportional to ruminal S^{2-} or HS^- concentration (1) and secondly, that S^{2-}, HS^-, and their derivatives are primary determinants of the fraction of ingested copper that becomes physiologically unavailable to ruminants. Among the sulfides of the essential metals, those of copper are unique in being stable at the lowest pH encountered in the digestive tract and, although susceptible to oxidation in vitro, apparently remain intact at the redox potentials encountered within the intestinal lumen (J. Price, J. K. Chesters, C. F. Mills, unpublished data), thus precluding utilization of copper therefrom.

The physiological nonavailability of copper when ingested as its sulfide has been apparent from the earliest studies of the role of copper as an essential nutrient (114) and has been confirmed in many subsequent investigations (3, 71). Intraruminal metabolites of both inorganic and organic dietary sources of sulfur metabolized via sulfide are also involved in the antagonistic action of molybdenum upon copper metabolism and thus in the etiology of molybdenum-induced copper deficiency of ruminants. The species specificity of the action of molybdenum as a copper antagonist (84) and the potentiating effects of a variety of dietary sulfur sources (120) accord with the view that the antagonistic potency of molybdenum is expressed most strongly in situations in which S^{2-} or HS^- are generated within the digestive tract by bacterial activity or are deliberately included in the diet.

The concept that the particular susceptibility of ruminants to molybdenum-induced copper deficiency reflects the intraruminal reaction of ingested molybdenum with endogenous sulfide or hydrosulfide to yield tetrathiomolybdate

$(MoS_4)^{2-}$, oxythiomolybdates $(MoO_nS_{4-n})^{2-}$, or their polymeric analogs, which subsequently react with copper to yield physiologically unavailable products, is being closely investigated. This suggestion originated, indirectly, from evidence that anomalies in the form of copper detected in the blood plasma of sheep receiving diets high in molybdenum (116) could be reproduced by intravenous infusion or in vitro addition to ovine plasma of ammonium tetrathiomolybdate $(MoS_4)^{2-}$ (29). The appearance in blood plasma of a variable fraction of total copper in a form that is insoluble in 5% wt./vol. trichloracetic acid (TCA), is now known to be contingent, initially, upon the association of either tetrathio- or oxythiomolybdates with the plasma albumen fraction and upon their subsequent reaction with copper entering plasma from either gut or tissues (11, 64, 74, 75, 86). The extent to which the thiomolybdates are absorbed from the gut of rats or ruminants to produce these and other anomalies in the form, distribution, and physiological availability of tissue Cu appears to be inversely proportional to the dietary Cu/Mo ratio (87, 123).

Several studies showed that tri- or tetrathiomolybdates form when populations of ovine or bovine rumen microorganisms are incubated in vitro in the presence of potential sources of S^{2-} or HS^- (e.g. SO_4^{2-}, cystine) and molybdate (4, 5, 30). However, evidence of the intraruminal formation in vivo of tri- or tetrathiomolybdate is either indirect (e.g. the appearance of anomalous, TCA-insoluble forms of molybdenum and copper in plasma after the absorption of thiomolybdates) or is confined to tentative spectrophotometric evidence of their presence in the soluble phase of rumen contents of animals receiving diets very high in molybdenum content (e.g. 100 mg Mo/kg) (30). Thus the relevance of the "thiomolybdate hypothesis" to the clearly established involvement of relatively lower dietary concentrations of dietary molybdenum ($\gtrsim$ 2 mg/kg) in the etiology of ruminant copper deficiency is not yet fully substantiated (82). Despite this, studies of the biochemical effects of the thiomolybdates clearly indicate that some, and particularly $(MoS_4)^{2-}$, are potent inhibitors both of the absorption of copper and of its redistribution within tissues (75, 86). Studies with rats, sheep or cattle indicate that antagonistic potency is related directly to the extent of thio-substitution within the series $(MoO_nS_{4-n})^{2-}$ whether this potency is assessed by monitoring inhibition of copper absorption, by the depression of cell cytosol content of copper in liver or kidney, by the inhibition of the activity of copper-dependent enzyme activity, or by monitoring the development of gross pathological defects preventable if additional copper is given (11, 86). It is noteworthy that correspondingly low dietary concentrations of molybdenum as molybdate (e.g. 6 mg/kg) or of reactive sulfide in most other forms have no significant antagonistic effect on copper metabolism when given to monogastric animals.

Evidence of inhibited copper absorption when tetrathiomolybdate is added to the diet of rats (96) or sheep (123) is fully compatible with its suggested role in

the etiology of molybdenum-induced copper deficiency in ruminants. However, systemic disturbances of copper utilization are almost always observed when MoS_4^{2-} is given to the nonruminant whereas effects of high dietary Mo in ruminants are usually confined to inhibition of Cu absorption. This casts doubt on whether or not intraruminal synthesis of free MoS_4^{2-} is an obligatory initial step in this antagonism (82). Further uncertainty arises from the observation (J. Price, J. K. Chesters, personal communication) that the physiological utilization of dietary copper by rats is inhibited more strongly by some molybdenum-containing fractions of ovine rumen and duodenal contents than by corresponding daily intakes of molybdenum as MoS_4^{2-}. Both these difficulties would be resolved if the relevant Mo-containing derivative formed by rumen bacterial activity proves to be a polymeric analogue of MoS_4^{2-} structurally incorporated into bacterial cells, more stable than the free monomeric ion at low gastric or abomasal pH, but retaining a high affinity for copper (82). Such aspects of the "thiomolybdate hypothesis" remain to be clarified.

Concurrent attempts to quantify and to predict the adverse effects of dietary Mo and S on the utilization of Cu by ruminants indicate that antagonistic potency is influenced markedly by the primary constituents of the diet. Thus Mo and S have much greater effect when present in fresh rather than dried forages or when added as supplements to cereal-based diets (121). The reasons for such variations may remain obscure until the variables influencing the intraruminal synthesis, stability, and antagonistic potency of the thiomolybdates and related Mo-S compounds are more clearly defined. Meanwhile, the action of MoS_4^{2-} as a copper antagonist is exploited very successfully in its therapeutic use to prevent copper intoxication in sheep (49, 50).

IMBALANCES INVOLVING IRON SUPPLY OR UTILIZATION

Zinc and the Metabolism of Iron and Copper

The recent proliferation of studies of metallothioneins in dietary imbalances of zinc, cadmium, and copper tends to obscure the frequently greater nutritional significance of interactions between these metals and iron. Although adverse effects of very high intakes of zinc, cadmium, or copper on the utilization or tissue storage of iron are widely recognized and well documented (45, 54, 76, 77, 106), recent studies indicate that a relatively lower intake of zinc from zinc-fortified conventional diets or therapeutic preparations can restrict the absorption and utilization both of iron and of copper (101). Thus, administration of 200 mg zinc per day to nonresponsive celiac patients reportedly induced anemia accompanied by hypocupremia (102). In human volunteers given iron and zinc supplements simultaneously, the absorption of constant doses of iron declined progressively as the Zn/Fe ratio of supplements increased from 0.4 to

2.5 (22). Fox and her associates (55, 56), in experiments with quail, investigated the effects of adding modest or excessive supplements of zinc to diets with iron and copper contents similar to those of human diets. In one group of studies, growth, hemoglobin, hematocrit, and liver iron and copper were frequently depressed when dietary zinc exceeded 125 mg/kg. In a second series, the adverse effects of 250- or 500-mg Zn/kg diet were overcome by increased supplementation with copper, provided that the dietary Zn/Fe ratio was >2. The merits and limitations of such animal models and the need for better understanding of the mechanisms involved in such interactions are fully discussed by these workers (40, 41).

Effects of Iron Deficiency on Trace Metal Metabolism

The above findings clearly indicate the need for general discretion in the fortification of diets with zinc if adverse effects on iron and copper utilization are to be avoided. It is becoming apparent moreover that the consequences of inorganic element imbalances may well be exacerbated if induced in subjects marginally or frankly deficient in iron. Thus, low dietary iron increases not only the efficacy of iron absorption but also that of lead (20, 36) zinc, cadmium, cobalt, and manganese (37, 53, 124, 125) and pre- and postnatal accumulation of lead by young rats is related, inversely, to the iron content of the maternal diet (17).

Particularly noteworthy in the context of suboptimal iron nutrition is preliminary evidence that a relatively mild degree of iron depletion enhances the uptake of other metals. Thus, in one study with human volunteers given ^{115m}Cd, cadmium absorption was enhanced from 2.6% of the dose in normal subjects to 7.5% in individuals with normal blood hemoglobin but low serum ferritin concentrations. In concurrent experiments with mice, iron deficiency increased deposition of cadmium in the duodenal mucosa, and enhanced both the transfer of cadmium from the gastrointestinal tract to body tissues and its deposition in kidneys (37). Rats given an iron-deficient diet for seven days, but showing no significant differences in hemoglobin or hematocrit, absorbed and retained from the diet 65% more ^{203}Pb in the carcass and, as in the above study with cadmium, retained more lead in intestinal tissue than animals of normal iron status (89).

Evidence of the effects of mild iron deficiency on absorption of lead by human subjects is conflicting. In one study with 10 human subjects among whom serum iron and serum ferritin ranged from normal to low, the efficiency of absorption of ^{59}Fe and of ^{203}Pb showed a strong positive correlation (127). [Although it has been suggested that this result arose from ineffective radioisotope discrimination during the counting of ^{59}Fe and ^{203}Pb activity (96), this criticism has been refuted (128)]. Whereas the mean fractional absorption of orally administered lead was 20% in normal subjects, it was 47% in subjects

classified as being of low iron status (127). Others (34, 35) found no such relationship between Fe status and Pb absorption in human subjects given isotopically labelled Pb and Fe. Although important procedural differences exist between these studies yielding conflicting data [such as the use of carrier-free Pb in the former (118) but not the latter (35)], different mechanisms of lead absorption may also exist in rodents and human subjects and may thus account for species difference in the influence of changes in iron status on lead retention (35). The relevance of this broad generalization appears questionable, however. Differences in response certainly do not extend to Fe/Cd relationships for which, both in mouse and man, there is evidence that a low iron status provokes increased absorption and tissue retention of cadmium (37).

Whatever mechanisms are responsible for the modulatory effects of changes in iron status on heavy metal absorption, it appears unlikely that they are attributable merely to nonselective incorporation of a range of metals into the mucosal carriers involved in the enhanced uptake of iron by iron-deficient subjects. For example, the effects of iron depletion upon metal absorption differ according to whether a low iron status is induced by dietary deficiency of this element or by phlebotomy (36). In contrast to a dietary deficiency of iron, reduction of iron stores by blood withdrawal failed to enhance absorption of lead or cadmium by mice even though that of cobalt, zinc, and possibly manganese were increased (36). The enhanced uptake of iron induced by transient anoxia is not accompanied by increased lead uptake (J. N. Morrison, personal communication), which again suggests that distinctly different absorptive mechanisms are involved.

It will remain difficult to assess the significance of iron-dependent effects upon metal absorption until more is known of the mechanisms involved and how these may differ among species. In the context of the nutrition of the newborn, it appears particularly important to substantiate indications from one study with rats that the modulatory effects of iron status upon metal absorption are not evident initially but develop during the postnatal suckling period (51). Variations in the degree of saturation of endogenous iron-binding ligands of dietary constituents such as milk may be relevant to the effects of iron status and supply on the absorption of other metals. In one recent study with rats (104, 105), enhanced absorption of lead achieved by giving liquid milk was simulated if a solid diet was given and apo-lactoferrin was administered by intragastric infusion. In contrast, iron-saturated lactoferrin did not enhance lead absorption. Zinc absorption was also enhanced by apo-lactoferrin, but less markedly than that of iron. Although, in addition to iron, apo-lactoferrin exhibits an affinity for zinc and for a range of other metals, it is not yet known whether they share a common binding site with iron or whether the above effects of apo-lactoferrin reflect nonselective metal binding by a protein with a known affinity for mucosal cell membranes. Evidence that in human milk (but not in cow's

milk) most of the zinc is bound to lactoferrin (2) suggests that similar differences could occur in the partition of other metals between milk proteins and could influence their absorption.

Effects of a High Iron Status

It is now becoming appreciated that the effectiveness of high dietary iron as an antagonist of copper utilization by ruminants has been grossly underestimated. Adverse effects of a high iron intake on copper metabolism were first suspected from ad hoc observations on the development of hypocupremia in cattle consuming irrigation waters rich in iron (22). Later work indicated that iron supplementation reduced hepatic copper storage in cattle and sheep (15, 52, 119), but the high levels of iron used in these experiments (1500–3200 mg Fe/kg) and failure to define, separately, the inhibitory effects of increments of sulfur intake arising from the supplementary source of Fe (as $FeSO_4$) delayed appreciation of the full significance of the Fe/Cu interactions.

A recent reinvestigation of this phenomenon indicated that only 250, 500, or 800 mg Fe/kg in the diet of cattle inhibited hepatic copper storage and depressed plasma copper, ceruloplasmin, and erythrocyte superoxide dismutase activities to levels indicative of severe copper depletion (60). It appears unlikely that high dietary iron enhances endogenous losses of copper since the depletion of hepatic copper stores is not accompanied by increases in biliary loss of copper (D. S. Graca, personal communication). Recent studies at the Rowett Institute indicate that this particular manifestation of the antagonistic effect of iron on copper metabolism is contingent upon the establishment of rumen function since high dietary iron does not inhibit the utilization of copper by unweaned calves.

Furthermore, there are indications from work with both rats (13) and sheep (113) that, like molybdenum, the antagonistic effect of iron is potentiated by the presence of sulfide in the digestive tract. Such evidence suggests that these and possibly other metallic elements may act as inhibitors of copper absorption in ruminants by a fundamentally similar mechanism (83). This could involve (*a*) sequestration within the rumen of (microbially produced) sulfide in the form of metal sulfides or polythio complexes stable at rumen pH (typically 6–7), and, after their passage to the more acid environment of the abomasum and proximal duodenum, either (*b*) their direct reaction with copper released from digesta by acid proteolysis or (*c*) reaction of such copper with S^{2-} arising from the decomposition of acid-unstable metal sulfides to form physiologically unavailable CuS or Cu_2S. The physiological feasibility of this concept was demonstrated recently by in vitro studies of the reactions of copper with a range of metals whose sulfides or thio complexes are less acid-stable and have higher solubility products than those of copper (83). Evidence of its biological relevance is currently confined to the findings that dietary supplements of iron

(113) or of molybdenum (44) increase the rumenal pool of potentially Cu-reactive sulfide and thus increase its flux to the duodenum, at which site copper is released and normally absorbed (8).

In marked contrast to the clinical response of ruminants equally severely depleted of blood and liver copper by the antagonistic action of high dietary molybdenum, the induction of a low copper status by high iron diets has not yet been found to induce clinical signs of copper deficiency (12, 60). Ostensibly relevant is the suggestion that, since copper depletion is accompanied by secondary defects in iron metabolism (43), some pathological sequelae of copper deficiency syndromes may well be ameliorated by increases in iron supply (129). In support of this suggestion, evidence was presented that inhibition of growth, protein synthesis, and hemopoiesis and the depression of hepatic ATP concentrations in copper-deficient rats were eliminated if dietary iron was increased from 25 to 40 or 80 mg Fe/kg. In contrast, increased provision of iron did not affect the decline of cytochrome oxidase activity nor the enhanced salt solubility of skin collagen induced by a deficiency of copper. While not all these claims have been reinvestigated, one recent study (130) fully substantiated the long-standing evidence (76) that increases in iron supply ameliorate the inhibitory effects of copper deficiency upon hemopoiesis, but it failed to substantiate the striking claim that additional iron rectifies the metabolic defect responsible for growth failure in copper deficiency (129).

Despite uncertainties as to the nature of pathological responses to a low copper status induced by iron excess, it is quite clear that concentrations of dietary iron commonly encountered by ruminants consuming forages contaminated with soil or receiving adventitious iron in mineral supplements are frequently sufficient to restrict utilization of copper (122).

Although adverse effects of high dietary iron on Cu metabolism are most strongly evident in ruminants, a reduced hepatic storage of copper in response to increases in the dietary Fe/Cu ratio has also been noted in guinea pigs (115) and in pigs (58). Other consequences of iron excess recently noted in nonruminants include inhibitory effects upon the absorption or metabolism of selenium and zinc. Thus, high tissue iron promoted by a high iron diet (>300 mg Fe/kg) inhibited the utilization of tissue selenium for synthesis of the selenoenzyme glutathione peroxidase (GSHpx) in livers and erythrocytes of rats (72). Since hepatic iron accumulation is a frequent feature of copper deficiency, this finding may well be relevant to evidence that deficiency of copper reduces the rate of postnatal increase in GSHpx activity in young mice and rats (103).

Neither from these investigations nor from those demonstrating the influence of a marginal or low iron status on the accumulation or absorption of lead and other metals is it yet possible to suggest mechanisms for these iron-dependent processes. It appears probable, however, from one recent study of the Fe/Zn

antagonism with human subjects (118) that Fe(II) may be a more potent inhibitor of zinc absorption than Fe(III). This conclusion is consistent with evidence that the adverse effects of a 2:1 Fe/Zn ratio on Zn absorption are exacerbated by dietary ascorbate. In contrast, heme Fe neither inhibits the absorption nor the systemic utilization of zinc in human subjects (117, 118). The importance of gaining an understanding of the mechanisms involved is again emphasized by the recent claim that, whereas inhibition of Zn absorption by Fe is clearly evident when both are administered in aqueous solution, the magnitude of the effect is not related to the previous Fe status of subjects and it is not apparent when the Fe/Zn ratio of solid diets is varied (111).

Recent studies with pregnant rats have also suggested that varying the Fe/Zn ratio of a solid diet between 0.8 and 3.7 does not influence the Zn status of the fetus (32, 33). We cannot yet exclude the possibility that sensitivity to a wide Fe/Zn ratio may change at differing stages of physiological development, but it appears more probable that antagonisms between Fe and Zn may have greater relevance to the efficacy of therapy with liquid supplements than to modest imbalances in the content of these elements in conventional diets.

Iron Metabolism in Deficiencies of Nickel and Lead

Less clearly established is the relevance of the forms of dietary iron to the effects of nickel deficiency on tissue iron storage. It has been suggested that the adverse effects of nickel deficiency on the utilization of a marginal supply of dietary iron are more strongly expressed when the latter is in the form of Fe(III) (95). While such differences in the form of iron may be related to conflicting observations on the effects of nickel on hepatic iron storage (69, 94, 95, 113), there is universal agreement that the anemia, depressed hematocrit, and, in some instances, the depressed growth rate induced by nickel deficiency in rats are related to inhibition of iron absorption and to secondary effects upon iron-dependent processes (69). Whether the depression of growth, blood hemoglobin, and serum iron and the concomitant increase in serum iron-binding capacity that develops during the induction of lead deficiency in rats result similarly from inhibition of iron absorption (66, 67) or from other defects reflecting an unknown role of lead has been speculated upon (68).

METABOLIC RESPONSES TO MULTIPLE DEFICIENCIES

Modified Responses to Zinc Deficiency

Familiar biochemical or clinical features of an individual inorganic element deficiency may be modified if concurrent deficiency of a second nutrient arises. Such situations are not uncommon both in agricultural practice (e.g. 46) and in the nutrition of socially deprived human communities (e.g. 47, 48). Ex-

perimental evidence that a concurrent deficiency of calcium ameliorates the teratogenic effects of zinc deficiency in rats (61) has been attributed to release and utilization of zinc from the resorbing skeleton. Other studies (78, 79) clearly illustrated that catabolic maternal responses to deficient protein or energy intake not only release maternal tissue zinc to permit fetal accretion of this element but can also promote sufficient maternal/fetal transfer to protect the fetus against a severe dietary deficiency of zinc. Similar maternal "buffering" against fetal deficiency of copper appears to be much less effective (79).

The influence of changes in the balance between dietary zinc and energy supply on the mobility of tissue zinc is also evident from investigations made on malnourished and zinc-deficient infants. Thus, restoration of normal growth following therapy with supplements (which included zinc) provoked a further rapid decline in plasma zinc to concentrations that were inversely proportional to rate of growth (47, 48). All the above observations have important implications both for the diagnostic interpretation of blood and tissue analyses and for the estimation of zinc and copper requirements.

Interrelated Effects of Selenium, Copper, and Manganese Deficiencies

Attention has been focussed upon responses to multiple deficiencies of nutrients whose biochemical roles, like those of copper and iron (38, 75) are closely related in metabolic sequences. Interrelationships likely to modify tissue susceptibility to pathological damage initiated by oxygen-derived free radicals have been investigated during the induction of single or multiple deficiencies of selenium, copper, and manganese in rats (99, 100). Effects on tissue peroxidation attributable directly or indirectly to changes in the activities of the enzymes (Se-) glutathione peroxidase and (Cu- and Mn-) superoxide dismutases have been assessed, as have the modifying effects of changes in dietary vitamin E supply.

A salient feature of these necessarily complex studies was that the potentiating effect of Se deficiency on lipid peroxidation was enhanced in some, but not all tissues, by concurrent deficiencies of copper or manganese. Tissues found particularly susceptible to such damage were frequently those inherently low in "non-Se-dependent glutathione peroxidase" (i.e. glutathione S-transferase) activity and those in which the activities of Cu- or Mn-dependent superoxide dismutases declined markedly in response to concurrent dietary deficiencies of copper or manganese. Particularly noteworthy in the light of present interest in the role of Se-lack as one variable associated with the etiology of infant cardiomyopathy in Keshan disease (18, 138, 139) were the observations in rats that a concurrent deficiency of Se strongly exacerbated the cardiomegaly,

ventricular fibrosis, and mortality induced by copper depletion and, that unlike most other syndromes associated with Se deficiency, this response was not eliminated by increases in vitamin E supply (100).

CONCLUSION

Studies of interactions among inorganic nutrients are slowly emerging from a period of intense preoccupation with the task of demonstrating their existence, regardless of the conditions required to produce such evidence. While this period was fruitful, its principal achievement was to show, not surprisingly, that many chemical reactions governing the mutual solubility and competition of structurally related inorganic elements for ligands can be demonstrated to occur in vivo if the animal is treated as a biological test tube! While the nutritional relevance of many such observations remains in doubt, it is quite clear that others reflect processes that are important determinants of the metabolism of these elements, which regulate requirements and the tolerance for inorganic imbalances, deficiencies or excesses. Although examples typifying the latter were considered in this review, it will have become apparent that knowledge of such interactions is largely qualitative and lacks the quantitative definition required if their full nutritional significance is to be assessed. Before this is possible it is essential to better understand the nature of the biochemical processes modifying the efficiency of absorption or tissue utilization of the elements involved, if only to clarify the nature of variables that directly or indirectly modify responses to changes in the dietary supply of potential antagonists. Thus, the difficulties that continue to be encountered in attempts to determine the significance of dietary phytate in the context of the zinc requirements of man are directly attributable to the low priority accorded until recently to studies of the variables that influence both the survival of this "inhibitor" in the digestive tract and its affinity for zinc within the intestinal lumen.

Similar uncertainties are delaying not only the reliable definition of tolerable dietary imbalances involving the elements molybdenum, copper, iron, zinc, and selenium but also the wider acceptance by toxicologists of the concept that nutrient supply, adequacy, or imbalance are significant determinants of heavy metal tolerance. While ad hoc nutritional experimentation has yielded much relevant and valuable information, further progress is certain to be contingent upon more detailed consideration of the physicochemical principles involved in the interactions responsible for modifying the absorption and metabolic fate of essential and potentially toxic trace elements. Additional justification of this argument is presented in two recent reviews covering both fundamental biochemical (131) and nutritional aspects (41).

Literature Cited

1. Bird, P. R. 1970. Sulphur metabolism and excretion studies in sheep. III. The effect of sulphur intake on the availability of copper to sheep. *Proc. Aust. Soc. Anim. Prod.* 8:212–18
2. Blakeborough, P., Salter, D. N. 1985. Zinc binding by lactoferrin and human milk. In *Trace Elements in Man and Animals* (TEMA-5), ed. C. F. Mills, I. Bremner, J. K. Chesters. Farnham Royal: C. A. B. Publ. In press
3. Bowland, J. P., Braude, R., Chamberlain, A. G., Glascock, R. F., Mitchell, K. G. 1961. The absorption, distribution and excretion of labelled copper in young pigs given different quantities as sulphate or sulphide, orally or intravenously. *Br. J. Nutr.* 15:59–72
4. Bray, A. C., Suttle, N. F., Field, A. C. 1982. The determination of thiomolybdates in continuous cultures of rumen microorganisms. *Proc. Nutr. Soc.* 41:66 (Abstr.)
5. Bray, A. C., Suttle, N. F., Field, A. C. 1982. The formation of tri- and tetrathiomolybdates in continuous cultures of rumen microorganisms and their adsorption onto "fibre". *Proc. Nutr. Soc.* 41:67 (Abstr.)
6. Bray, A. C., Till, A. R. 1975. Metabolism of sulphur in the gastrointestinal tract. In *Digestion and Metabolism in the Ruminant*, ed. I. W. McDonald, A. C. I. Warner, pp. 243–60. Armidale, NSW: Univ. New England
7. Bremner, I. 1981. The nature and function of metallothionein. In *Trace Element Metabolism in Man and Animals* (TEMA-4), ed. J. M. Howell, J. M. Gawthorne, C. L. White, pp. 637–42. Canberra: Aust. Acad. Sci.
8. Bremner, I., Davies, N. T. 1980. Dietary composition and the absorption of trace elements by ruminants. In *Digestive Physiology and Metabolism in Ruminants*, ed. Y. Ruckebusch, P. Thivend, pp. 409–27. Lancaster: MTP Press
9. Bremner, I., Mehra, R. K. 1983. Metallothionein: some aspects of its structure and function with special regard to its involvement in copper and zinc metabolism. *Chem. Scr.* 21:117–21
10. Bremner, I., Mills, C. F. 1981. Absorption, transport and tissue storage of essential trace elements. *Philos. Trans. R. Soc. London Ser. B*294:75–89
11. Bremner, I., Mills, C. F., Young, B. W. 1982. Copper metabolism in rats given di- or trithiomolybdates. *J. Inorg. Biochem.* 16:109–19
12. Bremner, I., Phillippo, M., Humphries, W. R., Young, B. W., Mills, C. F. 1983. Effects of iron and molybdenum on copper metabolism in calves. In *Trace Elements in Animal Production and Veterinary Practice*, ed. N. F. Suttle, R. G. Gunn, W. M. Allen, K. Linklater, G. Weiner, pp. 136–37. *Br. Soc. Anim. Prod. Occas. Publ. 7*
13. Bremner, I., Young, B. W., Mills, C. F. 1982. Effects of iron and sulphide on copper metabolism in rats. *Proc. Nutr. Soc.* 41:82A
14. Byrd, C. A., Matrone, G. 1965. Investigations of the chemical basis of the zinc-calcium-phytate interaction in biological systems. *Proc. Soc. Exp. Biol. Med.* 120:447–49
15. Campbell, A. G., Coup, M. R., Bishop, W. H., Wright, D. E. 1975. Iron-induced hypocuprosis. *Proc. NZ Soc. Anim. Prod.* 35:175–83
16. Carswell, A. J. P. 1981. *An investigation of the effect of calcium, phytic acid and fibre on zinc availability to young rats.* M.Sc. thesis. Univ. Aberdeen. 48 pp.
17 Cerklewski, F. L. 1980. Reduction in neonatal lead exposure by supplemental dietary iron during gestation and lactation in the rat. *J. Nutr.* 110:1453–57
18. Chen, X. S., Yang, G. Q., Chen, J. S., Chen, X. C., Wen, Z. M., Ge, K. Y. 1980. Studies on the relations of selenium and Keshan disease. *Biol. Trace Element Res.* 2:91–107
19. Cheryan, M. 1980. Phytic acid interactions in food systems. *CRC Crit. Rev. Food Sci. Nutr.* 13:297–335
20. Conrad, M. E., Barton, J. C. 1978. Factors affecting the absorption and excretion of lead in the rat. *Gastroenterology* 74:731–40
21. Cossack, Z. T., Prasad, A. S. 1983. Effect of protein source on the bioavailability of zinc in human subjects. *Nutr. Res.* 3:23–31
22. Coup, M. R., Campbell, A. G. 1964. The effect of excessive iron intake on the health and production of dairy cows. *NZ J. Agric. Res.* 7:624–38
22. Crofton, R. W., Gvozdanovic, D., Aggett, P. J. 1962. A study of the effect of zinc on iron absorption in man. *Proc. Nutr. Soc.* 41:17 (Abstr.)
23. Davies, N. T., Carswell, A. J. P., Mills, C. F. 1985. The effect of variations in dietary calcium intake on the phytate-zinc interaction in rats. See Ref. 2
24. Davies, N. T. 1982. Effects of dietary phytate on mineral availability. In *Di-*

etaryFiber in Health and Disease, ed. G. V. Vahouny, D. Kritchevsky, pp. 105–16. New York: Plenum
25. Davies, N. T., Nightingale, R. 1975. The effects of phytate on intestinal absorption and secretion of zinc and whole-body retentions of zinc, copper, iron and manganese in rats. *Br. J. Nutr.* 39:307–16
26. Davies, N. T., Olpin, S. E. 1979. Studies on the phytate:zinc molar contents in diets as a determinant of zinc availability to young rats. *Br. J. Nutr.* 41:591–603
27. Davis, P. N., Norris, L. C., Kratzer, F. H. 1962. Interference of soybean proteins with the utilization of trace minerals. *J. Nutr.* 77:217–23
28. DiSilvestro, R. A., Cousins, R. J. 1983. Physiological ligands for copper and zinc. *Ann. Rev. Nutr.* 3:261–88
29. Dick, A. T., Dewey, D. W., Gawthorne, J. M. 1975. Thiomolybdates and the copper-molybdenum-sulphur interaction in ruminant nutrition. *J. Agric. Sci.* 85: 567–68
30. El-Gallad, T. T., Mills, C. F., Bremner, I., Summers, R. 1983. Thiomolybdates in rumen contents and rumen cultures. *J. Inorg. Biochem.* 18:323–34
31. Erdman, J. W. Jr., Weingartner, K. E., Mustakas, G. C., Schmutz, R. D., Parker, H. M., Forbes, R. M. 1980. Zinc and magnesium availability from acid precipitated and neutralised soybean protein products. *J. Food Sci.* 45:1193–99
32. Fairweather-Tait, S. J., Payne, V., Williams, C. M. 1984. Effect of iron supplements on pregnancy in rats given a low-zinc diet. *Br. J. Nutr.* 52:79–86
33. Fairweather-Tait, S. J., Wright, A. J. A., Williams, C. M. 1984. Zinc metabolism in pregnant and lactating rats and the effect of varying iron:zinc in the diet. *Br. J. Nutr.* 52:205–13
34. Flanagan, P. R. 1980. Oral absorption of lead and iron. *Lancet* 2:699
35. Flanagan, P. R., Chamberlain, M. J., Valberg, L. S. 1982. The relationship between iron and lead absorption in humans. *Am. J. Clin. Nutr.* 36:823–29
36. Flanagan, P. R., Haist, J., Valberg, L. S. 1980. Comparative effects of iron deficiency induced by bleeding and a low iron diet on the intestinal absorptive interactions or iron, cobalt, manganese, zinc, lead and cadmium. *J. Nutr.* 110: 1754–63
37. Flanagan, P. R., McLellan, J. S., Haist, J., Cherian, M. G., Chamberlain, M. J., Valberg, L. S. 1978. Increased dietary cadmium absorption in mice and human subjects with iron deficiency. *Gastroenterology* 74:841–46
38. Forbes, R. M., Erdman, J. W. Jr. 1983. Bioavailability of trace mineral elements. *Ann. Rev. Nutr.* 3:213–31
39. Forbes, R. M., Erdman, J. W., Parker, H. M., Kondo, H., Ketelson, S. M. 1983. Bioavailability of zinc in coagulated soy protein (Tofu) to rats and effect of dietary calcium at a constant phytate:zinc ratio. *J. Nutr.* 113:205–10
40. Fox, M. R. S. 1978. Nutritional considerations in designing animal models of metal toxicity in man. *Environ. Health Perspect.* 25:137–40
41. Fox, M. R. S., Jacobs, R. M., Jones, A. O. L., Fry, B. E. Jr., Rakowska, M., et al. 1981. Animal models for assessing bioavailability of essential and toxic elements. *Cereal Chem.* 58:6–11
42. Freeland-Graves, J. H., Ebangit, M. L., Hendrikson, P. J. 1980. Alterations in zinc absorption and salivary sediment zinc after a lacto-ovo-vegetarian diet. *Am. J. Clin. Nutr.* 33:1757–66
43. Frieden, E. 1980. Caeruloplasmin: a multi-functional metalloprotein of vertebrate plasma. In *Biological Roles of Copper*, ed. D. Evered, G. Lawrenson, pp. 93–124. Amsterdam: Excerpta Medica
44. Gawthorne, J. M., Nader, C. J. 1976. The effect of molybdenum on the conversion of sulphate to sulphide and microbial protein-sulphur in the rumen of sheep. *Br. J. Nutr.* 35:11–23
45. Gipp, W. F., Pond, W. G., Kallfelz, F. A., Tasker, J. B., van Campen, D. R., et al. 1974. Effect of dietary copper, iron and ascorbic acid levels on hematology, blood and tissue Cu, Fe and Zn concentration and ^{64}Cu and ^{59}Fe metabolism in young pigs. *J. Nutr.* 104:532–41
46. Gleed, P. T., Allen, W. M., Mallinson, C. B., Rowlands, G. J., Sansom, B. F., et al. 1983. Effects of selenium and copper supplementation on the growth of beef steers. *Vet. Res.* 113:388–92
47. Golden, B. E., Golden, M. H. N. 1981. Plasma zinc, rate of weight gain and the energy cost of tissue deposition in children recovering from severe malnutrition on a cow's milk or soya protein based diet. *Am. J. Clin. Nutr.* 34:892–99
48. Golden, M. H. N., Golden, B. E. 1981. Effect of zinc supplementation on the dietary intake, rate of weight gain and energy cost of tissue depletion in children recovering from severe malnutrition. *Am. J. Clin. Nutr.* 34:900–08
49. Gooneratne, S. R., Howell, J. M., Gawthorne, J. M. 1981. Intravenous administration of thiomolybdate for the prevention and treatment of chronic copper poisoning in sheep. *Br. J. Nutr.* 46:457–68

50. Gooneratne, S. R., Howell, J. M., Gawthorne, J. M. 1981. An investigation of the effects of intravenous administration of thiomolybdate on copper metabolism in chronic Cu-poisoned sheep. *Br. J. Nutr.* 46:469–80
51. Gruden, N., Buben, M. 1981. Iron-59 and manganese-54 metabolism in suckling rats pretreated with iron-fortified milk. *Nutr. Rep. Int.* 24:943–50
52. Grun, M., Anke, M., Hennig, A., Seffner, W., Partschfeld, M., et al. 1978. Uberhohte orale Eisengaben an schafe. 2. Der einfluss auf den Eisen-, Kupfer-, Zinc und Mangangehalt verschiedener organe. *Arch. Tierernähr.* 28:341–47
53. 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. Pharmacol.* 56:384–89
54. Hamilton, D. L., Valberg, L. S. 1974. Relationship between cadmium and iron absorption. *Am. J. Physiol.* 227:1033–37
55. Hamilton, R. P., Fox, M. R. S., Fry, B. E. Jr., Jones, A. O. L., Jacobs, R. M. 1979. Zinc interference with copper, iron and manganese in young Japanese quail. *J. Food Sci.* 44:738–41
56. Hamilton, R. P., Fox, M. R. S., Tao, S. H., Fry, B. E. Jr. 1981. Zinc-induced anaemia in young Japanese quail ameliorated by supplemental copper and iron. *Nutr. Res.* 1:589–99
57. Harmuth-Hoene, A. E., Schelenz, R. 1980. Effects of dietary fibre on mineral absorption in growing rats. *J. Nutr.* 110:1774–84
58. Hedges, J. D., Kornegay, E. T. 1973. Interrelationships of dietary copper and iron as measured by blood parameters, tissue stores and feedlot performance of swine. *J. Anim. Sci.* 37:1147–54
59. Hill, C. H., Matrone, G. 1970. Chemical parameters in the study of *in vivo* and *in vitro* interactions of transition elements. *Fed. Proc.* 29:1474–81
60. Humphries, W. R., Phillippo, M., Young, B. W., Bremner, I. 1983. The influence of dietary iron and molybdenum on copper metabolism in calves. *Br. J. Nutr.* 49:77–86
61. Hurley, L. S., Tao, S.-H. 1972. Alleviation of teratogenic effects of zinc deficiency by simultaneous lack of calcium. *Am. J. Physiol.* 222:322–25
62. Jacobs, R. M., Jones, A. O. L., Fox, M. R. S., Lener, J. 1983. Effects of dietary zinc, manganese and copper on tissue accumulation of cadmium by Japanese quail. *Proc. Soc. Exp. Biol. Med.* 172:34–38
63. Kagi, J. H., Nordberg, M. 1979. *Metallothionein.* Basel: Birkhauser. 378 pp.
64. Kelleher, C. A., Ivan, M., Lamand, M., Mason, J. 1983. The absorption of labelled molybdenum compounds in sheep fitted with re-entrant cannulae in the ascending duodenum. *J. Comp. Pathol.* 93:81–92
65. Kelsay, J. L. 1981. Effect of diet fiber level on bowel function and trace mineral balances of human subjects. *Cereal Chem.* 58:2–5
66. Kirchgessner, M., Reichlmayr-Lais, A. M. 1981. Retention, absorbierbarkeit und intermeditaire verfugbarkeit von eisen bei alimentarem bleimangel. *Int. J. Vit. Nutr. Res.* 51:421–24
67. Kirchgessner, M., Reichlmayr-Lais, A. M. 1981. Changes in iron concentration and iron-binding capacity in serum resulting from alimentary lead deficiency. *Biol. Trace Element Res.* 3:279–85
68. Kirchgessner, M., Reichlmayr-Lais, A. M. 1982. Konzentrationen verschiedener stoffwechselmetaboliten in experimentellen bleimangel. *Ann. Nutr. Metab.* 26:50–55
69. Kirchgessner, M., Schnegg, A. 1980. Biochemical and physiological effects of nickel deficiency. In *Nickel in the Environment,* ed. J. O. Nriagu, pp. 635–52. New York: Wiley
70. Kirchgessner, M., Schwarz, F. J., Schnegg, A. 1982. Interactions of essential metals in human physiology. In *Clinical, Biochemical, and Nutritional Aspects of Trace Elements,* ed. A. S. Prasad, pp. 477–512. New York: Liss
71. Kline, R. D., Corzo, M. A., Hays, V. W., Cromwell, G. L. 1973. Related effects of copper, molybdenum and sulfide on performance, hematology and copper stores of growing pigs. *J. Anim. Sci.* 37:936–41
72. Lee, Y. H., Layman, D. K., Bell, R. R., Norton, H. W. 1981. Response of glutathione peroxidase and catalase to excess dietary iron in rats. *J. Nutr.* 111:2195–2202
73. Likuski, H. J. A., Forbes, R. M. 1965. Mineral utilisation in the rat: effects of calcium and phytic acid on the utilization of dietary zinc. *J. Nutr.* 85:230–34
74. Mason, J., Lamand, M., Hynes, M. J. 1983. ^{99}Mo metabolism in sheep after the intravenous administration of ^{99}Mo thiomolybdates. *J. Inorg. Biochem.* 19: 153–64
75. Mason, J., Lamand, M., Kelleher, C. A. 1982. The effects of duodenal infusion of tri- and dithiomolybdate on plasma copper and on the plasma diamine oxidase

activity of caeruloplasmin in sheep. *J. Comp. Pathol.* 92:509–17
76. Matrone, G. 1960. Interrelationships of iron and copper in the nutrition and metabolism of animals. *Fed. Proc.* 19:659–65
77. Matrone, G., Truggle, B. C., Ramsay, P. B. 1975. Studies on the elucidation of the *in vivo* interactions of high levels of dietary zinc on synthesis of liver ferritin. In *Proteins of Iron Storage and Transport in Biochemistry and Medicine*, ed. R. R. Crichton, pp. 347–58. Amsterdam: North-Holland
78. Masters, D. G., Keen, C. L., Lonnerdal, B., Hurley, L. S. 1983. Zinc deficiency teratogenicity: the protective effect of maternal tissue catabolism. *J. Nutr.* 113:905–12
79. Masters, D. G., Keen, C. L., Lonnerdahl, B., Hurley, L. S. 1983. Comparative aspects of dietary copper and zinc deficiencies in pregnant rats. *J. Nutr.* 113:1448–51
80. Mills, C. F. 1979. Trace elements in animals. *Philos. Trans. R. Soc. London B*288:51–63
81. Mills, C. F. 1980. Metabolic interactions of copper with other trace elements. See Ref. 43, pp. 49–69
82. Mills, C. F. 1982. Molybdenum as a biochemical antagonist of copper. In *Chemistry and Uses of Molybdenum*, ed. H. F. Barry, P. C. H. Mitchell, pp. 134–38. Ann Arbor: Climax
83. Mills, C. F. 1985. Influence of chemical speciation on the absorption and physiological utilisation of trace elements from the diet and environment. In *The Importance of Chemical Speciation in Environmental Processes*, ed. M. Bernhard, F. E. Brinkman. Berlin: Dahlem. In press
84. Mills, C. F., Bremner, I. 1980. Nutritional aspects of molybdenum in animals. In *Molybdenum and Molybdenum-Containing Enzymes*, ed. M. P. Coughlan, pp. 519–42. Oxford: Pergamon
85. Mills, C. F., Davies, N. T., Quarterman, J., Aggett, P. J. 1984. Metal interactions in the aetiology of trace element deficiency and toxicity. In *Proc. Int. Symp. Health Effects and Interactions of Essential and Toxic Elements, Lund, 1983*, ed. M. Abdulla. In press
86. Mills, C. F., El-Gallad, T. T., Bremner, I. 1981. Effects of molybdate, sulfide and tetrathiomolybdate on copper metabolism in rats. *J. Inorg. Biochem.* 14:189–207
87. Mills, C. F., El-Gallad, T. T., Bremner, I., Wenham, G. 1981. Copper and molybdenum absorption by rats given ammonium tetrathiomolybdate. *J. Inorg. Biochem.* 14:163–75
88. Morris, E. R., Ellis, R. 1980. Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semi-purified diets. *J. Nutr.* 110:1037–45
89. Morrison, J. N., Quarterman, J. 1981. Effect of sub-clinical iron deficiency on lead absorption. *Proc. Nutr. Soc.* 41:21 (Abstr.)
90. Nahapetian, A., Young, V. R. 1980. Metabolism of ^{14}C-phytate in rats: effect of low and high dietary calcium intakes. *J. Nutr.* 110:1458–72
91. National Research Council. 1978. *Nutrient Requirements of Laboratory Animals*. Washington, DC: Natl. Acad. Sci. 96 pp. 3rd (revised) ed.
92. Navert, B., Sandstrom, B., Cederblad, A. 1984. Dephytinisation of bran and its effect on zinc absorption in man. *Br. J. Nutr.* In press
93. Nelson, T. S., Kirby, L. K. 1979. Effects of age and diet composition on the hydrolysis of phytate phosphorus by rats. *Nutr. Rep. Int.* 20:729–34
94. Nielsen, F. H. 1980. Effect of form of iron on nickel deprivation in the rat: plasma and liver lipids. *Biol. Trace Element Res.* 2:199–210
95. Nielsen, F. H., Shuler, T. R. 1981. Effect of form of iron on nickel deprivation in the rat: liver content of copper, iron, manganese and zinc. *Biol. Trace Element Res.* 3:245–56
96. Oberleas, D., Muhrer, M. E., O'Dell, B. L., Kuntner, L. D. 1961. Effects of phytic acid on zinc availability in rats and swine. *J. Anim. Sci.* 20:56–62
97. Obizoba, I. C. 1981. Zinc and copper metabolism of human adults fed combinations of corn, wheat, beans, rice and milk containing various levels of phytate. *Nutr. Rep. Int.* 24:203–10
98. Paskiss-Hurlburt, A. J., Tanaka, Y., Skoryna, S. C., Moore, W. Jr., Stara, J. F. 1977. The binding of lead by a pectic polyelectrolyte. *Environ. Res.* 14:128–40
99. Paynter, D. I. 1980. The role of dietary copper, manganese, selenium and vitamin E in lipid peroxidation in tissues of the rat. *Biol. Trace Element Res.* 2:121–35
100. Paynter, D. I., Martin, G. B. 1980. Investigations into combined dietary deficiencies of copper, selenium and vitamin E in the rat. *Biol. Trace Element Res.* 2:175–91
101. Prasad, A. S., Brewer, G. J., Schoomak-

er, E. B., Rabbani, P. 1978. Hypocupraemia induced by zinc therapy in adults. *J. Am. Med. Assoc.* 240:2166–68
102. Porter, K. G., McMaster, D., Elmes, M. E., Love, A. H. G. 1977. Anaemia and low serum copper during zinc therapy. *Lancet* 2:774
103. Prohaska, J. R., Gutsch, D. E. 1983. Development of glutathione peroxidase activity during dietary and genetic copper deficiency. *Biol. Trace Element Res.* 5:33–45
104. Quarterman, J. 1983. The role of milk and lactoferrin in the absorption of essential and toxic metals. *Proc. 4th Spurenelement Symp*, ed. M. Anke, W. Baumann, H. Braunlich, pp. 187–93. Leipzig: Karl Marx Univ.
105. Quarterman, J. 1984. Milk, lactoferrin and heavy metal absorption. *Proc. Nutr. Soc.* 42:45 (Abstr.)
106. Rama, R., Planas, J. 1981. Dietary cadmium effect on iron metabolism in chickens. *Biol. Trace Element Res.* 3:169–83
107. Roberts, A. H., Yudkin, J. 1960. Dietary phytate as a possible cause of magnesium deficiency. *Nature* 185:823–25
108. Rose, H. E., Quarterman, J. 1984. Phytic acid and heavy metal uptake and depletion. *Proc. Nutr. Soc.* 43:6 (Abstr.)
109. Rose, H. E., Quarterman, J. 1985. The influence of dietary phytic acid and calcium on lead absorption in the rat. See Ref. 2
110. Sandstrom, B., Arvidsson, B., Cederblad, A., Bjorn-Rasmussen, E. 1980. Zinc absorption from composite meals. The significance of wheat extraction rate, zinc, calcium and protein content in meals based on bread. *Am. J. Clin. Nutr.* 33:739–45
111. Sandstrom, B., Davidsson, L., Cederblad, A., Lonnerdal, B. 1985. Effect of inorganic iron on the absorption of zinc from a test solution and a composite meal. See Ref. 2
112. Sathe, V., Krishnamurthy, K. 1953. Phytic acid and absorption of iron. *Indian J. Med. Res.* 41:453–61
113. Schnegg, A., Kirchgessner, M. 1976. Zur interaktion von nickel, eisen, kupfer und zink. *Arch. Tierernähr.* 26:543–49
114. Schultze, M. O., Elvehjem, C. A., Hart, E. B. 1936. Further studies on the availability of copper from varius sources as a supplement to iron in haemoglobin formation. *J. Biol. Chem.* 115:453–57
115. Smith, C. H., Bidlack, W. R. 1980. Interrelationships of dietary ascorbic acid and iron on the tissue distribution of ascorbic acid, iron and copper in female guinea pigs. *J. Nutr.* 110:1398–1408
116. Smith, B. S. W., Wright, H. 1975. Effect of dietary Mo on Cu metabolism. *Clin. Chim. Acta* 62:55–63
117. 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. *J. Clin. Nutr.* 34:475–82
118. Solomons, N. W., Pineda, O., Viteri, F., Sandstead, H. H. 1983. Studies on the bioavailability of zinc to humans: mechanisms of the intestinal interaction of nonheme iron and zinc. *J. Nutr.* 113:337–49
119. Standish, J. F., Ammerman, C. B., Palmer, A. Z., Simpson, C. F. 1971. Influence of dietary iron and phosphorus on performance, tissue mineral composition and mineral absorption in steers. *J. Anim. Sci.* 33:171–78
120. Suttle, N. F. 1975. The role of organic sulphur in the copper-molybdenum-sulphur interrelationship in ruminant nutrition. *Br. J. Nutr.* 34:411–20
121. Suttle, N. F. 1983. The nutritional basis for trace element deficiencies in ruminant livestock. See Ref. 12, pp. 19–25
122. Suttle, N. F., Abrahams, P., Thornton, I. 1984. The role of a soil × dietary sulphur interaction in the impairment of copper absorption by ingested soil in sheep. *J. Agric. Sci.* 103:81–86
123. Suttle, N. F., Field, A. C. 1983. Effects of dietary supplements of thiomolybdates on copper and molybdenum metabolism in sheep. *J. Comp. Pathol.* 93:379–89
124. Thomson, A. B. R., Olatunbosun, D., Valberg, L. S. 1971. Interrelationship of intestinal transport system for manganese and iron. *J. Lab. Clin. Med.* 78:642–55
125. Thomson, A. B. R., Shaver, C., Lee, D. J., Jones, B. L., Valberg, L. S. 1971. Effect of varying iron stores on site of intestinal absorption of cobalt and iron. *Am. J. Physiol.* 220:674–78
126. Turnlund, J. R., Swanson, C. A., King, J. 1983. Copper absorption and retention in pregnant women fed diets based on animal and plant proteins. *J. Nutr.* 113:2346–52
127. Watson, W. S., Hume, R., Moore, M. R. 1980. Oral absorption of lead and iron. *Lancet* 2:236–37
128. Watson, W. S., Moore, M. R., Hume, R. 1980. Oral absorption of lead and iron. *Lancet* 2:699
129. Weisenberg, E., Halbreich, A., Mager, J. 1980. Biochemical lesions in copper-deficient rats caused by secondary iron deficiency. *Biochem. J.* 188:633–41
130. Williams, D. M., Scott, K. F., Green, B. G. 1983. Hepatic iron accumulation in

copper deficient rats. *Br. J. Nutr.* 50: 653–60
131. Williams, R. J. P. 1981. Physico-chemical aspects of inorganic element transfer through membranes. *Philos. Trans. R. Soc. London Ser. B*294:57–74
132. Wise, A. 1982. Blood lead levels after chronic feeding to mice of lead acetate with calcium phytate in the diet. *Bull. Environ. Contam. Toxicol.* 29:550–53
133. Wise, A. 1984. Dietary Ca influences location and extent of phytate hydrolysis in the rat intestine. See Ref. 2
134. Wise, A. 1983. Dietary factors determining the biological activity of phytate. *Nutr. Abstr. Rev. Rev. Clin. Nutr.* 53: 791–806
135. Wise, A., Gilburt, D. J. 1981. Binding of cadmium and lead to the calcium-phytate complex in vitro. *Toxicol. Lett.* 9:45–50
136. Wise, A., Gilburt, D. J. 1982. In vitro competition between calcium phytate and the soluble fraction of rat small intestine contents for cadmium, copper and zinc. *Toxicol. Lett.* 11:49–54
137. Wise, A., Richards, C. P., Trimble, M. L. 1983. Phytase hydrolysis in the gastrointestinal tract of the rat followed by phosphorus-31 Fourier transform nuclear magnetic resonance spectroscopy. *Appl. Environ. Microbiol.* 45:313–14
138. Xu, G.-L., Hong, S.-Y. 1984. Keshan disease and selenium deficiency. See Ref. 85
139. Zhu, L. 1981. Keshan disease. See Ref. 7, pp. 514–17