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PHYSIOLOGICAL LIGANDS FOR COPPER AND ZINC

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INTRODUCTION

The chemical properties of copper and zinc render these elements both extremely beneficial and extremely deleterious to biological systems. Not surprisingly then, animals possess specific mechanisms for channeling the proper amount of copper and zinc to the correct physiological location in order to carry out specific functions. Most aspects of these mechanisms are poorly under-

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stood. However, probably all of the many ligands with which these metals associate in some way influence the delicate balance between essentiality and toxicity. For the purpose of this review, ligands are defined as neutral or negatively charged molecules that surround a central metal via specific binding forces.

ABSORPTION

Differences in bioavailability of trace metals in foods must relate to chelation effects with specific dietary and endogenous components. During the course of digestion these associations may be substantially modified. The acidic conditions of the stomach contents and their subsequent neutralization in the intestine will create opportunities for potential ligands to undergo changes in extent of ionization that will affect stability of metal-ligand complexes.

Copper

DIETARY FACTORS The copper absorption process is not well understood. A general feeling has persisted that some copper is actively absorbed bound to certain amino acids. The main evidence for this hypothesis has come from Kirchgessner & Grassmann (110). They found that copper added to animal diets as amino acid complexes produced greater liver copper accumulation than did equivalent amounts of copper as $CuSO_4$. L-amino acids were more effective than D-isomers, which implied that specific transport mechanisms were involved. In contrast, Marceau et al (125) observed the same absorption rates of ^{64}Cu whether or not the metal was bound to histidine. They assumed that the acidic environment of the stomach disassociated the amino acid–metal complex causing a large dilution of the small amount of histidine. Various high protein diets fed to laboratory animals and humans produced variable effects on copper balance (47, 54, 84, 132). Perhaps the different types of amino acids and short amino acid polymers resulting from protein digestion all influenced copper absorption differently. Furthermore, the different amounts of dietary copper and means of assessing copper absorption utilized in the various studies probably also contributed to the lack of a unified concept.

The distinct phases of copper absorption have not been extensively investigated. For example, copper transport across the brush border membrane has not been investigated directly, although amino acids may provide ligands for this process. The interference of zinc with copper absorption was believed to be an intracellular antagonism. However, since kinetics of zinc transport by the brush border membrane are altered by zinc deficiency, and this deficiency enhances copper absorption, and high levels of lumen zinc depress copper uptake into mucosa (135), a common membrane component could exist. Dietary

ligands that favor the absorption of zinc may hinder copper transport and vice versa.

Nonprotein components of diet probably also influence copper absorption. Yet the extent to which any given component actually would affect copper absorption is difficult to predict. A concept that not all copper complexes are absorbed to the same degree dates back to 1936. Schultze et al (177) found that copper associated with citrate, phosphate, and gluconate reversed anemia induced by copper deficiency, but copper sulfide and copper porphyrin did not. Later, Mills (140) found that copper present in fresh herbage caused more liver copper accumulation than did copper given as $CuSO_4$. Other studies suggested that $CuNO_3$, $CuCO_3$, Cu-Oxalate, and Cu-EDTA are all better absorbed than $CuSO_4$, (25, 110). On the other hand, phytates (47), ascorbic acid (98, 200), and the combination of molybdenum plus sulfur (44, 194) seem to inhibit copper absorption. Some high fiber diets might also provide binding ligands that result in low copper uptake by the intestine (109). Low molecular weight compounds formed during digestion of foods may influence the extent of absorption. The enhancement of copper accumulation with copper chelators such as NTA (108) suggests copper may be transported across some membranes as an intact complex.

However, the results of many of these studies are difficult to interpret. A large number involve repletion from a copper deficiency state and many include the administration of extremely high doses of copper and the dietary component in question. It is not known how findings of this type can be applied to relatively healthy humans consuming fairly moderate levels of copper and other dietary components. Many studies relied also on indirect measurements of copper absorption. For instance, liver copper accumulation has been one parameter used to demonstrate ascorbic acid antagonism of copper absorption in chicks (98). However, ascorbic acid has been shown recently to exert a postabsorption effect on copper metabolism in chicks (48) that could also influence liver copper concentrations. A further understanding of the copper absorptive process will be fostered by future experiments directed at specific aspects of copper absorption (e.g. intestinal membrane vesicles and intestinal perfusions) where systemic effects can be distinguished from specific mechanisms.

ENDOGENOUS LIGANDS Various secretions may play a role in the homeostatic control of copper absorption. Bile, the major vehicle of copper excretion, contains some compound(s) that seem to inhibit reabsorption of excreted copper (83). The size and nature of these compounds are still under investigation. In addition, rat pancreas is capable of secreting a protein that may limit copper absorption (105). Human gastric and salivary secretions

contain low molecular weight compounds capable of binding copper (82). However, when ^{64}Cu was mixed with these secretions and introduced intraduodenally into rats, ^{64}Cu absorption was not any greater than that obtained from ^{64}Cu-acetate (82). Still, these secretions might help solubilize dietary copper, thus indirectly aiding absorption.

It has generally been assumed that proteins in intestinal mucosal cells in some manner regulate copper absorption, yet relatively few experimental data have been reported in this area. Most of the work focuses on intestinal metallothionein (see below for a discussion of this protein, which is found in various organs). Whether metallothionein comprises an essential component of copper absorption is not known, but this protein may help protect against copper toxicity. Sheep seem to show both a limited ability to synthesize intestinal metallothionein and a high susceptibility to copper poisoning (174). At present, it is not known if copper alters the metallothionein gene expression in intestinal cells. Further work is needed for better understanding of this and other aspects of copper absorption.

Zinc

DIETARY FACTORS Evidence from both animal experiments and clinical studies with humans suggest zinc absorption is homeostatically regulated. The exact nature of the mechanism involved is not clear, but a number of control points may be involved. The first phase of zinc absorption involves transport across the brush border membrane surface. This transport step is probably a passive diffusion process. Undoubtedly, a multitude of intraluminal factors influence this phase. Recent experiments on zinc transport by isolated brush border membrane vesicles from rat intestine established that the transport velocity is an inverse function of dietary zinc status; thus a homeostatic mechanism at the membrane level appears to be activated by a diminished supply of zinc in the diet (135). ATP does not stimulate zinc transport in this system, suggesting that a diffusion type of process is more likely. This raises the question of exactly how zinc traverses the brush border membrane.

Clearly, any factor(s) in the diet that would increase the availability of zinc to membrane receptors would enhance the bioavailability (absorbability) of zinc. Unfortunately, it is not clear whether zinc traverses the brush border membrane and/or the membranous substructure of enterocytes as a free ion, or as a chelated complex. Evidence tends to support transport as a complex. For example, experiments utilizing a variety of approaches showed that EDTA has a stimulatory influence on zinc absorption (73, 146, 205). Suso & Edwards proposed that Zn and EDTA were transported from the intestinal lumen to the portal circulation as an intact complex (192, 193). The EDTA binding constant for Zn is particularly strong (128, 206), suggesting that the readily absorbable EDTA molecule increases the absorption of zinc by acting as a cotransported

species. Recently, Oestreicher & Cousins reinvestigated the absorption promoting effect of EDTA using an isolated, vascularly perfused intestine model (147). Of the moieties tested for direct effects on absorption, i.e. histidine, cysteine, methionine, tryptophan, picolinate, glutathione, citrate, and EDTA, only EDTA was an effective ligand in promoting transfer of zinc from the lumen to the portal blood supply (a criterion for absorption with this technique). Similarly, zinc transport by brush border vesicles of rat intestine was not increased by reduced glutathione, picolinate, or citrate (137). Collectively, these data suggest that these substances have no special relationship to zinc uptake.

It is generally believed that zinc in human milk is more bioavailable than zinc from cow's milk (discussed below in section on acrodermatitis enteropathica). Recently, considerable attention focused on low molecular weight zinc binding ligands as an explanation for this marked difference in bioavailability. Lonnerdahl and co-workers proposed, based mainly on chromatographic evidence, that the principal low molecular weight zinc binding ligand in human milk is citric acid (120). However, evidence from a variety of experimental approaches has not substantiated that citrate promotes zinc absorption. These include the isolated perfused intestine (147), brush border membrane vesicles from intestine (137), in vivo intubation (79, 104), and repletion-type feeding studies with zinc and citrate (102). In contrast, Evans & Johnson identified picolinic acid, a metabolite of tryptophan metabolism, as the low molecular weight ligand in human milk that binds most of the zinc (60). They obtained evidence with intact animal models suggesting picolinic acid increases zinc absorption (61). However, when tested in a variety of systems, picolinic acid was found to be without an effect (72, 102, 104, 137, 147).

These roles of citrate and picolinate have drawn criticism. Holt suggests that binding of large amounts of Ca^{2+} and Mg^{2+} to caseins in milk compromise the amount of Zn^{2+} that can be bound to other ligands (99). The inference is made that the binding of metals to milk proteins, rather than the presence of specific low molecular weight ligands, is the primary factor involved. May et al proposed, through a computer simulation of the interactions between Zn^{2+} and the low molecular weight ligands in milk, that at low picolinate concentrations this ligand binds most of the zinc (130). However, it is unlikely that picolinic acid is present in milk in very high concentrations since it is cytotoxic (70). Furthermore, May et al have also pointed out that at physiological pH, Zn-citrate is a negatively charged ligand and thus not likely to be taken up by intestinal cells. This is corroborated by lack of an effect of citrate on the uptake of zinc by brush border membrane vesicles in vitro (137) or in vivo (104).

Collectively, the available evidence does not suggest convincingly that a unique ligand in milk accounts for the greater bioavailability of zinc from human milk. Cousins & Smith proposed, instead, that the type of protein in

milk, its relative digestibility, and the amount of zinc that is potentially available to enter a free or low molecular weight chelated pool will determine the bioavailability of zinc in milk from different species (39). There is a substantial free amino acid content in milk (78), and Martin et al suggested that glutamic acid may be as important a ligand for zinc in the absorption process as others that have been proposed (129). Giroux & Prakash evaluated a variety of zinc-ligand mixtures for an effect on absorption using an increase in serum zinc as an index. Lysine, cysteine, and glycine had some effect (79). Protein restriction reduces zinc absorption but high dietary protein appears to increase it (24, 36, 182, 202), further suggesting a link between amino acids as ligands for zinc in the intestinal lumen. A definitive answer regarding dietary factors that provide ligands that influence absorption awaits delineation of the mechanism of absorption. The various biochemical and physiological aspects of zinc absorption have been reviewed in detail elsewhere (36, 182).

ENDOGENOUS LIGANDS The gastrointestinal tract may produce binding ligands that influence zinc absorption. However, at present, no single moiety represents a clear link to zinc uptake. The notion that a binding ligand may be involved stems principally from the observation that ^{65}Zn binds to extracts of tissues from the intestinal mucosal cells and/or pancreas. Early experiments of a number of laboratories showed that ^{65}Zn would co-migrate with low molecular weight species when soluble intestinal cell preparations were fractionated by gel filtration chromatography (87, 193, 201). A concept that these intestinal zinc ligands were involved in the absorption process was further developed, since the extent of absorption appeared to be directly correlated with binding (62, 167). A number of attempts were made to partially characterize this species. Amino acids (87), a polypeptide (175), prostaglandin E_2 (183), and picolinic acid (61) have been suggested. In contrast to these findings, Cousins et al demonstrated that intestinal cell preparations will yield differing chromatographic separations when extreme care in tissue handling and chromatography is not exercised (34, 38). Protein degradation is primarily responsible for generating lower molecular weight zinc binding species separable by chromatography; this has been confirmed (121). Moreover, anomalous ligand binding as based upon gel filtration chromatographic evidence was found to occur when the zinc content of the intestinal cells and/or lumen was appreciably altered. This latter finding suggests that experiments where exogenous ^{65}Zn is added in vitro may not provide a realistic measure of actual Zn^{2+} binding. Thus, interpretation of these types of experiments must be approached cautiously (36, 182).

Pancreatic and biliary secretions are known to contain zinc. This complement of zinc accounts for some, but not all, of the endogenous fecal zinc content (139). Pancreatic zinc secretion is reduced in zinc deficiency (191), but

the role of the pancreas in the zinc absorption process per se is not clear (62, 157). Zinc binding ligands in pancreatic secretions appear to be of relatively high molecular weight (23, 122, 156), and possibly include newly secreted pancreatic proteins (156). In contrast, when ^{65}Zn is added to pancreatic extracts or secretions in vitro, radioactivity may bind to species that separate on gel filtration chromatography as low molecular weight ligands (62). This points out the problems associated with in vitro labeling as noted above. In bile, zinc binds principally to low molecular weight components (122). Inhibitor studies indicate one of these biliary ligands is glutathione (2). The elucidation of the possible role these zinc ligands play in zinc absorption and/or excretion awaits clarification.

SERUM COMPONENTS

Copper Transport

Serum copper can be found in two major pools. One is tightly bound to the protein ceruloplasmin, and the other is associated less tightly with albumin and some low molecular weight compounds, especially histidine (97). In the postprandial state, ceruloplasmin accounts for over 90% of the total serum copper in man (86). In some animals this percentage is lower (186). These two copper pools do not exchange metal with one another (188). Recently, ceruloplasmin secretion by isolated liver parenchymal cells into culture medium has been shown to be stimulated by glucocorticoids (210). This suggests that this copper ligand responds to physiological stimuli that lead to elevated glucocorticoid levels. These cellular events could alter the amount of copper that enters the systemic circulation.

At one time it was generally assumed that the more labile copper pool represented the major source of copper for tissue metalloenzymes. However, Owen (150) found that intravenous injections of radioactive copper into rats did not produce maximal accumulation of radioactivity in extrahepatic tissues until after the emergence of labeled ceruloplasmin from the liver. Although copper that had accumulated inside tissue cells was not distinguished from that of blood trapped within organs, this study still lent credibility to the concept of a copper transport role for ceruloplasmin. Campbell et al (19) also utilized radioactive copper to provide some additional evidence for this proposal.

Several nonisotopic studies generated indirect evidence that ceruloplasmin functions as the major copper transporting agent for extrahepatic tissues. Hsieh & Frieden (101) intravenously injected a number of copper complexes into copper-deficient rats to evaluate their effect on restoring cytochrome C oxidase activity. In several organs ceruloplasmin restored activity to a greater extent than $CuCl_2$, copper-albumin, or copper-histidine. The six-day period over which this study was conducted is much greater than the half-life of ceruloplas-

min in rats and direct effect of protein-bound copper is equivocal (126). Harris & DiSilvestro (48, 92) correlated increased activity of the aortic copper enzyme lysyl oxidase with elevated ceruloplasmin levels in developing chicks undergoing a number of different treatments. Presently, no single study conclusively proves that ceruloplasmin represents the major source of copper for extrahepatic tissue metalloenzymes, but the bulk of the current evidence favors this hypothesis. Nonceruloplasmin serum copper may have multiple functions. It is fairly certain that copper recently absorbed from the digestive tract is transported to the liver by albumin and other nonceruloplasmin agents (89, 127, 209). The latter ligands may also play a major role in copper storage and excretion. Radioactive copper ions introduced into the serum rapidly accumulate in the liver and kidney (19, 50), which both seem to function in copper storage and excretion (14, 22, 187). Interestingly, dog albumin does not bind copper and this animal is very susceptible to copper toxicity, suggesting albumin copper is an essential feature of copper transport (50).

Nonceruloplasmin copper may also contribute to some extent to extrahepatic tissue copper uptake (19, 50). This contribution may be particularly high under certain circumstances such as Wilson's disease, where ceruloplasmin levels are low (21), or when certain chelators reach high levels in the serum. The tripeptide glycylhistidyllysine (GHL) (159), a serum protein called histidine-rich glycoprotein (143), and bioflavonoids (49) have all been discussed in regard to the latter. However, a major role in copper transport for any of these remains highly speculative. GHL has received the most attention because of its ability to stimulate growth and ^{64}Cu uptake in cultured hepatoma cells (158). Still, GHL has not been shown to increase activity levels of any copper metalloenzymes nor have GHL-copper complex levels and turnover times in serum been established as sufficient to make a major contribution to tissue copper utilization in vivo.

Zinc Transport

Total hematic zinc is distributed between erythrocytes and serum in a ratio of approximatley 8:1 or 9:1. The binding sites for zinc in erythrocytes have not been well characterized, but carbonic anhydrase may provide the principal binding ligand (197). Some zinc may become bound to membrane sites in acting as a stabilizer of these macromolecular structures (9). On the other hand, plasma zinc is the component through which the flux of metabolically active zinc is routed (6, 30, 52, 56, 117, 133, 153, 169).

Proteins provide the principal ligands for zinc transport in plasma. The distribution of zinc among these is fairly uniform among animal species, with albumin representing the primary binding ligand (11, 31, 152, 161). Albumin-bound zinc correlates well with the total serum zinc content and seems to be the zinc binding–plasma fraction that alters most during acute disease (66). Zinc bound to $alpha_2$-macroglobulin has an unclear metabolic function, but the

amount of zinc associated with this ligand does not appear to be influenced by metabolic changes. It has been suggested that transferrin-bound zinc is physiologically relevant (11), but albumin binds zinc more strongly (26). A function for transferrin in the portal transport of zinc was proposed (59). However, when the transfer of zinc from the intestinal lumen to the portal vein was studied directly, via an isolated perfused intestine, albumin was the ligand with which newly absorbed metal was transported (179, 180). Furthermore, the extent of absorption was shown to be influenced by the albumin content, not by transferrin (179). The circulating level of albumin may, therefore, be an important determinant of the rate of zinc absorption. Recent studies with isolated rat liver parenchymal cells have shown zinc is accumulated from media containing only amino acids and albumin (63, 64). These findings further support the concept that albumin is the functional ligand for zinc transport between the intestine and the liver. Albumin is also a potentially important factor in copper transport in the portal system, and characterization studies suggest that the copper and zinc binding sites are not the same (145).

A relatively small portion of the zinc in plasma is bound to amino acids, particularly histidine and cysteine (97, 145). The physiological significance of this association remains unclear, but zinc can likely be taken up by some cells as low molecular weight ligands (e.g. brain). Similarly, zinc excretion by the kidney may involve complex formation with amino acids. The bulk of these low molecular weight complexes must be reabsorbed by the kidney to preclude excessive urinary zinc loss (204, 212).

INTRACELLULAR LIGANDS AND METABOLISM

Copper Metalloenzymes

Copper has been considered to be an essential trace metal since 1928 when Hart et al (95) discovered that anemia prevention required copper as well as iron. Since then, copper has been implicated in such diverse functions as pigmentation, keratinization of wool, myelination of the spinal cord, bone formation, reproduction, cardiac function, and connective tissue maturation (198). The contribution of copper to these processes probably results from this metal's role in the structure and catalytic activity of metalloenzymes. Although these enzymes function in many diverse biological pathways, the catalytic action of the bound copper seems to be fairly similar in many cases. Most reactions catalyzed by copper metalloenzymes have been postulated to involve electron transfers and enzymatic binding of molecular oxygen. Owen recently reviewed much of the current knowledge about copper metalloenzymes (151). The binding of copper to these macromolecular ligands may influence copper metabolism, particularly in the course of their turnover, but that has not been investigated extensively.

Besides acting as a ligand in copper transport, ceruloplasmin also displays enzymatic activity. The physiological functions proposed for this activity include oxidation of biogenic amines, destruction of superoxide radicals, and iron mobilization (75). The latter proposal received the most attention. Ceruloplasmin oxidizes Fe^{2+} to Fe^{3+} in vitro with ceruloplasmin-bound Cu^{2+} being reduced to Cu^{1+} as a concomitant process (43). Several positive correlations between hemoglobin values, serum iron concentrations, and ceruloplasmin levels have also been established (160, 171). These observations led to the proposal that ceruloplasmin oxidizes iron originating from the liver and other organs as a necessary prerequisite for iron incorporation into the serum transport protein transferrin. Not all depressions in ceruloplasmin levels result in anemia, but reduction in ceruloplasmin levels must be extremely drastic before iron metabolism becomes impaired (27, 75, 162).

Most of the so-called "amine oxidases" also contain copper. One of these enzymes, lysyl oxidase, received considerable attention. Several prominent symptoms of copper deficiency result from diminished lysyl oxidase activity. This enzyme initiates crosslink formation in collagen and elastin that is necessary for proper connective tissue maturation and bone formation (93). In addition, aortic lysyl oxidase in chicks provides an excellent model system for studying tissue copper metabolism. Enzyme activity is virtually absent in eight-day-old chicks deficient in copper, but starts returning within hours following a single injection of $CuSO_4$ (90).

The complete range of functions performed by copper-containing enzymes has not been fully determined. Some enzymes have been proposed to contain copper, but the evidence in these cases is not conclusive. Examples include tryptophan-2,3 dioxygenase (103), mitochondrial monoamine oxidase (184), and butyryl coenzyme A dehydrogenase (190). Probably, some copper-containing metalloenzymes still remain to be discovered. It should also be realized that the functional influence of several well-studied copper enzymes such as ceruloplasmin, lysyl oxidase, and superoxide dismutase might be broader than once thought (91, 123, 211). In addition, certain enzymes containing copper could be involved in immunocompetence and the inflammatory response (163, 211). Finally, several copper metalloproteins have been found that have not shown a discernable enzyme activity, or the physiological significance of the enzyme activity is unclear. Such ligands include pink copper protein found in human erythrocytes (166), albocuprein I, II, and neurocuprein—all identified in human brain (76, 176)—and diamine oxidase, studied mainly in pig kidney (141).

Zinc Metalloenzymes

Since zinc was first documented as a dietary essential, the most widely accepted molecular role for zinc relates to its function as a constituent of a

variety of metalloenzyme systems. More than 70 enzymes, from every enzyme class, have been shown to require zinc. The metal provides structural integrity to the protein and/or participates directly in the reaction at the catalytic site. These enzymes are particularly relevant because they are involved in key biochemical functions. Salient examples of zinc metalloenzymes include DNA and RNA nucleotidyltransferases, alkaline phosphatase, and carbonic anhydrase (51). The seemingly divergent functions of zinc as a nutrient—particularly for tissue growth, bone formation, skin integrity, cell-mediated immunity, and generalized host defense—are related to the plethora of zinc metalloenzymes associated with these functions (29). These enzymes provide the bulk of the ligands to which zinc binds in cells. Exactly how these associations vary as a function of zinc status has not been clearly defined, however. Binding affinities for zinc vary; thus during a diminished dietary zinc supply, zinc from higher affinity sites of metalloproteins will be retained, while zinc in sites of other ligands may be removed and used to satisfy other cellular needs. The extent to which a ligand will lose zinc depends upon the geometry of the nitrogen, oxygen, and/or sulfur atoms that compromise the binding site.

Kirchgessner and associates reviewed the abundant literature on the relationship of dietary zinc to zinc binding to enzyme ligands (111, 112). Alkaline phosphatase activity of serum, intestine, bone and pancreatic carboxypeptidase A activity have consistently been shown to decrease during experimental zinc deficiency. In contrast, alcohol dehydrogenase, aldolase, carbonic anhydrase, glutamic, lactic, and malic dehydrogenase activities have been shown to be refractory to or inconsistently influenced by this deficiency. Bettger & O'Dell (9) emphasized this lack of response. They suggested that the role of zinc in membrane structure could be paramount to that related to metalloproteins.

These metalloenzymes form distinct ligands for zinc in tissues and serum. How this generalized property relates to or contributes to the highly regulated intermediary metabolism of zinc is not clear. When the cytosol fraction of a liver cell is examined, the majority of the zinc is protein bound. This distribution could, of course, be altered by processes where synthetic rate is less than degradation rate. However, the majority of the zinc metalloenzymes are probably produced by constitutive synthesis; thus a constant supply of these ligands is provided. An interesting model for the potential involvement of zinc with an enzyme has been proposed for fructose 1,6-bisphosphatase. Zn^{2+} inhibits the activity of this gluconeogenic enzyme under specific in vitro conditions (196), and in situ activity could be regulated by binding to an intracellular chelator such as histidine (154). The results could also be interpreted as a classical zinc metalloenzyme ligand system (8), but the concept that intracellular zinc could act as an allosteric regulator of physiological processes is of interest because the flux of plasma zinc into some cells may be altered by various stimuli (discussed

above). Unfortunately, zinc metabolism does not have a counterpart similar to that of ceruloplasmin in copper metabolism.

Metallothionein

Metallothionein is a metal binding ligand that appears to be involved in the metabolism of both copper and zinc. The physical properties of this metalloprotein have been extensively reviewed (106). All mammalian metallothioneins have a highly conserved primary structure of 61 amino acids, and 25–35% of these are cysteine. Up to 5–7 g-atoms of metal are bound per molecule. Data from ^{113}Cd-NMR suggest cadmium, and presumably zinc, are bound in two clusters via a tetrahedral arrangement of the thiolate ligands (149). Usually, copper and zinc are the predominant metals bound, the absolute concentrations being dependent upon many factors. In humans, fetal liver metallothionein contains primarily copper (173). In adult liver, zinc is the predominant metal associated with the protein (17). Similar developmental changes are observed in experimental animals (7).

Since metallothionein has no apparent enzymatic function (106), it must be viewed as an intracellular binding ligand. A particularly unique feature of this ligand is its inducibility by physiological stimuli. Its biosynthetic regulation is influenced by the dietary zinc supply. Repletion of zinc deprived rats results in an increase in hepatic and intestinal metallothionein mRNA activity, followed by a transient increase in zinc bound in these tissues to nascent metallothionein (133, 136). Cadmium and zinc injections (3, 53, 195) produced similar results. Food restriction will induce metallothionein (13, 168), as will physical stress, bacterial infection (148, 181), and copper administration (15). Through experiments with isolated liver cells, we established that glucocorticoids induce metallothionein gene expression and increase the amount of zinc taken up by hepatocytes and bound to metallothionein (63, 64). Insulin or glucagon were required for this effect. Glucocorticoids also induce the protein in a variety of cultured cells (107, 131). It was also possible to correlate increases in metallothionein mRNA activity to hepatic zinc redistribution and reductions in plasma zinc content in intact animals (56). We proposed, based upon these data, that regulation of metallothionein gene expression accounts for part of the altered hepatic zinc metabolism observed during stress and acute diseases (33, 35, 41). In hepatocytes, these changes seem to occur without appreciable copper redistribution to metallothionein (209), although copper administration does increase metallothionein mRNA activity (138).

Regulation of hepatic metallothionein is multihormonal in nature. Etzel & Cousins observed that glucagon administration produces a transient increase in metallothionein-bound zinc (57). This effect is synergistic with glucocorticoids and it does not require any changes in metallothionein mRNA. The action of glucagon in the maintenance of hepatic glucose production in response to

various physiological stimuli is well documented (199). In this regard, the responsiveness of metallothionein-bound zinc to glucagon could result from release of muscle zinc during catabolism of muscle protein and subsequent influx of the metal into the circulation and uptake by the liver. Apparently, that influx is not sufficient to activate increased metallothionein gene expression. In view of the effect zinc may have on carbohydrate metabolism (111), a physiological ligand for this metal that responds to gluconeogenic hormones is logical. It also implies that caution must be exercised when interpreting experiments with metallothionein that may involve inanition as a variable. The latter can lead to release of hormones that might shift zinc dynamics in a way that negates effects on metallothionein caused by dietary changes or stresses. Immunostaining for metallothionein in hepatocytes suggests an intracellular function, but since the protein is also localized in hepatic sinusoids, an excretory role, at least when cadmium is bound, cannot be precluded (45).

The function of metallothionein in the intestine is not clear. We proposed some time ago that it is involved in regulating absorption in response to acute changes in zinc status (33, 167). Others suggested it is not related to this process (104). We base our proposal on the fact that acute changes in dietary zinc increase the expression of metallothionein gene in intestinal cells, as measured by changes in mRNA activity (136). This is accompanied by enhanced binding of zinc to the protein. Changes in absorption of zinc can be correlated to this short-term shift in synthetic rate (136, 180). The isolated, perfused intestine has been shown to respond homeostatically to previous zinc treatment. Starcher et al suggested that intestinal metallothionein directly correlates to absorption of zinc (185), but since metallothionein synthesis is lowest in zinc-deficient rats, this seems less likely. McMaster et al reported that glucagon will stimulate zinc absorption by isolated, perfused rat intestine when directly added to the vascular perfusate (134). This suggests experimental manipulation that leads to glucagon secretion could obscure a fine control on absorption. The mutual antagonism between intestinal copper and zinc absorption has been related to metallothionein (88).

Present evidence suggests long-term changes in zinc and/or copper absorption are less closely related to metallothionein. Without a constant stimulus, expression of the metallothionein gene declines, and synthesis of the mRNA returns to a lower steady-state rate. Changes in absorption are not affected by this protein. As a unifying hypothesis, it is possible that metallothionein is an essential ligand that zinc must interact with during absorption. Only when the zinc load is excessive is the gene expressed, which results in more metallothionein synthesis and temporary intracellular retention. As discussed above, the endogenous secretion of zinc may provide a longer-term regulatory phase of absorption (208). Moreover, intestinal brush border membranes from rats of altered zinc status exhibit altered zinc transport (135), suggesting a

control point at cellular uptake may exist. The plasma flux of zinc associated with stress could also profoundly influence these mechanisms, both in an acute and long-term way. For example, Endotoxin has been reported to enhance ^{65}Zn absorption in intact rats (155). An alternative function for intestinal metallothionein could relate to excretion. Antibodies to metallothionein localize in the surface columnar cells of the villi and Paneth cells, suggesting that the protein is concentrated in these areas of the intestine (45). These results are compatable with roles for either absorption or excretion.

Metallothionein was first isolated from kidney (106); however, its functions in this organ have not been widely investigated. Both zinc and copper bind to this ligand in kidney cells under normal dietary conditions. Cadmium is also bound when exposure to environmental sources of this metal occurs (40). Furthermore, numerous reports suggest that kidney damage could be related in some cases to accumulation of cadmium and copper as metallothionein (65, 74, 106). Administration of mercurial diuretics and mercury salts also leads to binding of mercury to this renal ligand (164). Metallothionein could be involved in interorgan metal transport, since it is taken up by kidney. Immunological evidence suggests it is present in the serum, and in some cases within renal tubules (16, 28, 74). The evidence strongly suggests this binding ligand is produced in the kidney. Zinc and cadmium administration increases kidney metallothionein mRNA activity (53, 195). Similarly, in liver, this mRNA is associated with free polyribosomes (178), a finding that suggests metallothionein is principally an intracellular protein and is not secreted into plasma in meaningful quantities. On the other hand, zinc injections will increase its plasma content significantly (77). Since the plasma distribution of Zn and possibly Cu can change during illness, metallothionein may serve as a defense mechanism against appreciable zinc loss associated with a shift to plasma ligands that could pass into the glomerular filtrate (195). Renal handling of zinc responds to glucagon (203), a hormone that influences metallothionein-bound zinc in the liver (57). Glucagon may also influence renal metallothionein. Several reviews on metallothionein in relationship to metal metabolism appeared recently (12, 17, 74).

Equally relevant to synthesis of this physiological ligand is degradation. We established that metallothionein is degraded in relationship to the metal bound. Zinc metallothionein is degraded with a t½ of 18–20 hours (67). Zinc seems to leave the protein during proteolysis. In contrast, cadmium metallothionein is degraded more slowly. The protein moiety has a t½ of 3.7 days, but cadmium seems to be rebound to nascent polypeptides yielding an exceedingly long t½ (68). Thus when metallothionein is degraded, zinc is available for cellular efflux while cadmium is retained via rebinding to nascent apoprotein. Binding affinity for cadmium is greater than zinc (106). Ligand binding also influences the degradation of metallothionein observed under in vitro conditions. Cad-

mium metallothionein is more resistant than zinc metallothionein to proteolytic attack by either neutral or lysosomal proteases (69). Copper metallothionein is not degraded in an in vitro system utilizing lysosomal proteases (96). These degradation data help to explain why some metals, principally copper and cadmium, tend to accumulate when bound to metallothionein.

UPTAKE AND EFFLUX

The mechanism by which copper and zinc traverse cellular membranes is not known. Thus any discussion regarding the uptake and efflux of these metals by cells must be based on what is known about how the external and internal cellular environments influence these processes. Our understanding of binding to intracellular ligands has begun to provide a framework upon which biochemical and physiological observations can be integrated. Copper and zinc content of cells can be altered by changes in transport from the serum, ligand binding, and/or efflux.

Zinc Uptake and Efflux

As discussed in sections above, zinc enters intestinal cells by an unknown uptake mechanism. Menard & Cousins reported that the brush border membrane is sensitive to changes in dietary zinc and transport kinetics are altered by a diminished supply (135). Efflux from enterocytes may depend on the availability of plasma albumin (179). The extent of binding to metallothionein as a mechanism for retention of intracellular zinc from either the lumen or plasma direction of flux seems to be more related to acute changes in dietary zinc status (36, 136). Zinc efflux in the plasma to lumen direction could also involve metallothionein. The whole concept of endogenous zinc efflux via the intestinal route requires further study. Kowarski et al showed that net zinc flux across everted jejunal segments in the serosal to mucosal direction is greater than is likely to occur by passive mechanisms (113). Efflux of endogenous zinc via the pancreatic secretions is considerable and appears to be an important factor in regulating overall zinc balance on a chronic basis (191, 208), although the contribution of these secretions to homeostasis is equivocal (157).

In the past decade, a tremendous increase in studies on the metabolism at the cellular level has occurred. Cox & Ruckenstein reported that glucocorticoids would increase zinc accumulation in a variety of culture cell lines (42). Failla & Cousins demonstrated that zinc accumulation in isolated rat liver parenchymal cells was increased by glucocorticoids and that metallothionein synthesis accounted for this increase (64). Uptake was a temperature and energy-dependent saturable process. Maximum uptake occurred at about the normal plasma zinc concentration (63). The selective stimulation was not observed without insulin or glucagon. This work has been reviewed in detail (41, 209).

These principles have been directly related to observations with intact rats. Etzel et al found that glucocorticoids increase liver metallothionein zinc, but not total liver zinc (56). This is concomitant with enhancement in metallothionein mRNA and reduction in plasma zinc. The latter could result from increased cellular uptake or increased excretion. Since cells respond to glucocorticoids, increased hepatic accumulation is the most plausible explanation. Efflux of this additional complement of zinc then depends upon the degradation rate of the intracellular ligands to which zinc is bound. For example, zinc metallothionein degrades five times more rapidly than cadmium metallothionein, the latter being the metal more likely to be retained (see above). Normally, as the stimuli for its induction are absent, little metallothionein is found in cells. The mechanism that influences cellular protein turnover also regulates zinc metalloprotein degradation in other tissues. The availability of ligands in plasma, e.g. albumin and amino acids, may also play a significant role in cellular zinc efflux and in this way influence overall zinc balance.

The question of how cells alter uptake and efflux of trace metals such as copper and zinc is difficult to answer. Undoubtedly, a balance exists to maintain requisite needs of the extracellular fluids and intracellular compartments (37). Changes in the dietary supply, physiological stimuli, and malfunction of uptake and efflux mechanism influence this balance. The latter provides the metabolic basis for copper, and zinc accumulation disease.

Copper Uptake and Efflux

The process by which cells take up and release (efflux) copper has been defined only in general terms. For example, the liver has been postulated to be somewhat unique in its handling of this metal. The liver probably takes up more nonceruloplasmin copper than any other organ (19, 127, 150). This process, which may proceed by active transport (209), probably occurs at an especially high rate following copper absorption from the GI tract. Once inside the liver cells, copper is incorporated initially into several protein ligands, especially metallothionein (127, 209). Thus copper efflux back into the serum is inhibited. The liver may also take up significant amounts of copper through endocytosis of ceruloplasmin destined for degradation (85).

The two major pathways of hepatic copper efflux are unique to the liver. In this organ, copper is incorporated into bile components (187), which targets the metal for fecal excretion. Probably, the amount of copper available to extrahepatic tissues is primarily regulated by the liver through the processes of ceruloplasmin synthesis, biliary copper excretion, and copper incorporation into metallothionein. Weiner & Cousins have shown through immunological techniques that epinephrine and glucagon will stimulate the incorporation of copper into ceruloplasmin while glucocorticoids and sex hormones increase secretion of this copper binding ligand from hepatocytes (210). Inhibition of de

novo protein synthesis increases copper accumulation in these cells apparently by inhibiting synthesis of ceruloplasmin (210). Sternlieb (187) recently reviewed liver copper metabolism in detail.

Kidney uptake and efflux of copper also seem to play a role in copper homeostasis. As noted earlier, this organ takes up copper from both ceruloplasmin and nonceruloplasmin sources with substantial amounts of copper bound to metallothionein as an apparent end point. The kidney also releases some copper into urine, although this process usually accounts for only a small percentage of total copper excretion (22). Urinary excretion may also be responsive to hormonal status, particularly insulin and glucagon ratios (116).

Ceruloplasmin has been proposed to be the primary source of copper for extrahepatic metalloenzymes. However, the mechanism for transferring the copper tightly held by this ligand to tissue cells is a matter of conjecture. Frieden (75) suggests ceruloplasmin binds to a cell membrane receptor that either induces the release of copper or brings the whole protein into the cell by endocytosis. In either case, one or more reducing agents could facilitate copper release by generating the more labile Cu^{1+} state in ceruloplasmin. Alternatively, copper release from ceruloplasmin could be increased by partial degradation of the protein, either inside or outside the cell. Very few experimental data are actually available on this subject. However, Harris et al (94) found evidence for the existence of a receptor in chick aortic and heart membranes that is specific for ceruloplasmin. Also, immunochemical studies by Linder & Moore implied that ceruloplasmin might ultimately enter cells (118).

The copper uptake pathway may be directly linked to the insertion of copper into metal-free apoenzyme ligands. Chicks deficient in copper, which show low ceruloplasmin levels, have little aortic lysyl oxidase activity despite fairly normal aortic copper concentrations (4). Activation of this enzyme in vitro requires an intact tissue, fully oxygenated media, and active protein synthesis (165).

Much work remains to be done concerning cellular copper uptake and efflux. Virtually nothing is known about the latter process in extrahepatic cells. It is fairly certain, though, that the pathways of copper uptake and efflux tend to insure delivery of copper to the appropriate enzymes while preventing excessive cellular copper accumulation. Two disorders of copper metabolism—Wilson's disease and Menkes' syndrome—are believed to involve alterations in these pathways.

DISEASES OF COPPER AND ZINC METABOLISM

Wilson's Disease

Wilson's disease provides a dramatic example of how copper overaccumulation is as deleterious as copper deficiency. This recessively inherited disorder

(5) is characterized by low ceruloplasmin levels and biliary copper excretion, but high copper accumulation in the liver, kidney, brain, and sometimes the cornea of the eye (187). The major genetic defect is thought to occur in the liver, which may represent the first site of copper overaccumulation (81). Later, following liver damage, copper might then be released to the blood where it could be picked up by other tissues. The kidney would be expected to be particularly susceptible to copper overload owing to its seemingly large capacity to absorb nonceruloplasmin copper and low cellular turnover rate (14, 19, 150).

The low ceruloplasmin and high liver copper levels closely resemble neonatal conditions. Perhaps Wilson's disease represents a failure to establish the adult pattern of copper metabolism (55).

Ceruloplasmin synthesis and release, plus excretion of copper with the bile, normally comprise the main routes of copper elimination from the liver (187). Initially, reduced ceruloplasmin synthesis was thought to be responsible for Wilson's disease. However, Wilson's disease patients have substantial amounts of apoceruloplasmin, a low copper form of the protein, in their serum (20). It was later proposed that hepatic metallothionein from Wilson's disease patients had an abnormally high binding constant for copper (58), but this conflicted with earlier findings (142). Much of the current attention in this area focuses on a possible defect in the lysosomes, organelles that are believed to play a role in biliary copper excretion (189).

If Wilson's disease is detected before irreversible organ damage occurs, it can be treated fairly successfully with a low-copper diet and administration of d-penicillamine (207). This potent copper chelator seems to sequester excess copper and stimulate urinary excretion of the metal.

Menkes' Syndrome

In contrast to Wilson's disease, Menkes' syndrome is characterized by symptoms greatly resembling copper deficiency. This X-linked recessively inherited disorder has no known cure. Usually the disease is terminal at a very early age, presumably due to low activities of copper-dependent enzymes. This inherited copper deficiency disease results from malabsorption of copper that accumulates to a large extent in intestinal mucosal cells (46). However, skin fibroblasts cultured from Menkes' syndrome patients also display increased capacity to take up copper, but very little ability to release (efflux) the metal (80). Despite high intracellular copper concentrations, these cells show little activity of the copper-dependent enzyme lysyl oxidase (172). Mottled mouse mutants, which display many metabolic similarities to humans suffering from Menkes' syndrome, accumulate abnormally high levels of injected ^{64}Cu in most tissues other than liver (124).

Camakaris et al (18) suggest several possible molecular defects that could be involved in Menkes' syndrome: *(a)* production of a metallothionein with an abnormally high affinity for copper; *(b)* increased synthesis and/or lowered degradation rates for metallothionein; *(c)* an altered intracellular copper transport protein that binds the metal rather weakly, thus allowing copper accumulation and subsequent induction of metallothionein; *(d)* a defect in the efflux of copper from cells or from a cell compartment.

Several investigators have presented evidence against the first hypothesis (10, 115). In contrast, some evidence has been obtained that metallothionein synthesis rates might be unusually high in lymphoblasts cultured from Menkes' syndrome patients (170). It is not known whether this possible elevation in synthesis causes or results from abnormal copper accumulation in the cells. The latter possibility received some support from microscopic evidence that seems to indicate the presence of copper deposits beneath the membranes of cells extracted from Menkes' syndrome patients (100). It is not known whether the copper might be trapped upon entering, or upon leaving the cell. However, the speed at which copper enters lymphoblasts cultured from Menkes' syndrome patients and accumulates in metallothionein (170) would seem to make the latter hypothesis more likely. More precise characterization of the metabolic defect occurring in Menkes' syndrome will require further research.

Acrodermatitis Enteropathica

Diseases that have unique characteristics directly attributable to zinc are rare. An exception is the zinc-responsive syndrome, acrodermatitis enteropathica (AE) in humans and a similar condition in cattle, lethal trait A-46 (adema disease). Both are characterized by pronounced skin lesions, diarrhea, growth failure, and diminished immune function. Zinc malabsorption has been demonstrated in both conditions (71, 119). Prior to the first successful treatment of AE with supplemental zinc (144), the antifungal agent diodoquin (5,7-di-iodo-8-hydroxyquinoline) was used as a treatment. This compound is now recognized as a zinc binding ligand, and its ability to develop complete clinical remission is ascribed to that property (1). Human milk has been shown to alleviate the clinical signs of AE and a number of ligands have been proposed to account for the therapeutic effect of this food. As noted above, the issue has not been completely resolved. Evans & Johnson proposed that the picolinic acid content of human milk was responsible for the amelioration of AE lesions (60). The putative effect of pancreatic enzyme preparations in treatment of AE was postulated to be due to the picolinate content (114). However, picolinic acid did not override the metabolic block in zinc absorption in calves with adema disease, but hydroxyquinolines that are effective in treating AE were also effective in augmenting intestinal zinc absorption in these calves (72). The

documented toxicity of picolinate (70) detracts from any therapeutic value it may have. Lonnerdal and coworkers suggested that citric acid is the ligand that accounts for the therapeutic value of human milk in AE (120). When tested in bioassays, however, neither citrate nor picolinate significantly improve zinc absorption. When tested in systems that measure zinc absorption or transport directly (isolated vascularly perfused intestine, and brush border membrane vesicles), neither citrate nor picolinate have a demonstrable effect (137).

Collectively, the data suggest that the very nature of the ligands to which zinc is bound in human milk may not explain its therapeutic value. Rather as emphasized by Cousins & Smith (39), the protein composition of the milk may affect zinc bioavailability. The more digestible proteins of human milk provide ligands that may facilitate absorption. The nature of the metabolic block in AE is not known, but the intestine appears to be in a net secretory phase (1). Mounting evidence suggests that in AE, zinc can enter the intestinal lumen from the cells. Therefore, while ^{65}Zn uptake into mucosal cells may be low in AE (1), the metabolic lesion involved could involve excessive binding to an intracellular ligand such as metallothionein (32). Elucidation of the exact molecular defect awaits a more definitive understanding of the basic mechanism of trace element absorption.

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Literature Cited

1. Aggett, P. J., Delves, H. T., Harries, J. T., Bangham, A. D. 1979. The possible role of diodoquin as a zinc ionophore in the treatment of acrodermatitis enteropathica. *Biochem. Biophys. Res. Commun.* 87:513–17
2. Alexander, J., Aaseth, J., Refsvik, T. 1981. Excretion of zinc in rat bile—a role of glutathione. *Acta Pharmacol.* 49: 190–94
3. Anderson, R. D., Weser, U. 1978. Partial characterization and translation in vitro of rat liver metallothionein messenger ribonucleic acid. *Biochem. J.* 175:841–52
4. Balthrop, J. E., Dameron, C. T., Harris, E. 1982. Comparison of pathways of copper metabolism in aorta and liver. *Biochem. J.* 204:541–48
5. Bearn, A. G. 1957. Genetic and biochemical aspects of Wilson's disease. *Am. J. Med.* 22:747–57
6. Beisel, W. R. 1977. Zinc metabolism in infection. In *Zinc Metabolism: Current Aspects in Health and Disease,* ed. G. J. Brewer, A. S. Prasad, pp. 155–76. NY: Liss
7. Bell, J. V., Waalkes, M. P. 1982. Role of hepatic metallothionein during perinatal development in the rat. In *Biological Roles of Metallothionein,* ed. E. C. Foulkes, pp. 99–111. NY: Elsevier/North-Holland
8. Benkovic, P. A., Camperelli, C. A., De Maine, M., Benkovic, S. J. 1978. Binding and kinetic data for rabbit liver fructose-1,6-bisphosphatase with Zn^{2+} as cofactor. *Proc. Natl. Acad. Sci. USA* 75:2185–89
9. Bettger, W. J., O'Dell, B. L. 1981. A critical physiological role of zinc in the structure and function of biomembranes. *Life Sci.* 28:1425–38
10. Bonewitz, R. F. J., Howell, R. R. 1981.

Synthesis of a metallothionein-like protein in cultured human skin fibroblasts: relation to abnormal copper distribution in Menkes' disease. *J. Cell. Physiol.* 106:339–48
11. Boyett, J. D., Sullivan, J. F. 1970. Distribution of protein-bound zinc in normal and cirrhotic serum. *Metabolism* 19: 148–57
12. Brady, F. O. 1982. The physiological function of metallothionein. *Trends Biochem. Sci.* 7:143–45
13. Bremner, I., Davies, N. T. 1975. The induction of metallothionein in rat liver by zinc injection and restriction of food intake. *Biochem. J.* 149:733–38
14. Bremner, I., Hoekstra, W. G., Davies, N. T., Williams, R. B. 1978. Renal accumulation of (copper,zinc)-thionein in physiological and pathological states. In *Trace Element Metabolism in Man and Animals-3*, ed. M. Kirchgessner, pp. 44–51. Germany: Freising
15. Bremner, I., Hoekstra, W. G., Davies, N. T., Young, B. W. 1978. Effect of zinc status of rats on the synthesis and degradation of copper induced metallothionein. *Biochem. J.* 174:883–92
16. Bremner, I., Young, B. W. 1976. Isolation of (copper,zinc) thioneins from pig liver. *Biochem. J.* 155:631–35
17. Buhler, R. H. O., Kagi, J. H. R. 1974. Human hepatic metallothioneins. *FEBS Lett.* 39:229–34
18. Camakaris, J., Danks, D. M., Phillips, M., Herd, S., Mann, J. R. 1982. Copper metabolism in Menkes' syndrome and mottled mouse mutants. In *Inflammatory Diseases and Copper*, ed. J. R. J. Sorenson, pp. 85–96. Clifton, NJ: Humana
19. Campbell, C. H., Brown, R., Linder, M. C. 1981. Circulating ceruloplasmin is an important source of copper for normal and malignant animal cells. *Biochim. Biophys. Acta* 678:27–38
20. Carrico, R. J., Deutsch, H. F., Beinert, H., Orme-Johnson, W. H. 1969. Some properties of an apoceruloplasmin like protein in human serum. *J. Biol. Chem.* 244:4141–46
21. Cartwright, G. E., Markowitz, H., Shields, G. S., Wintrobe, M. M. 1960. Studies on copper metabolism. XXIX. A critical analysis of serum copper and caeruloplasmin concentrations in normal subjects, patients with Wilson's disease, and relatives of patients with Wilson's disease. *Am. J. Med.* 28:555–63
22. Cartwright, G. E., Wintrobe, M. M. 1964. The question of copper deficiency in man. *Am. J. Clin. Nutr.* 15:94–110
23. Casey, C. E., Hambidge, K. M., Walravens, P. A. 1979. Zinc binding in human duodenal secretions. *J. Pediat.* 95: 1008–10
24. Chan, W., Calhoun, N. R., Howard, M. P., Smith, J. C. Jr. 1979. ^{65}Zinc retention, plasma and bone zinc concentration: affected by level of dietary protein in rats. *Fed. Proc.* 38:1998 (Abstr.)
25. Chapman, H. L. J., Bell, M. C. 1963. Relative absorption and excretion by beef cattle of copper from various sources. *J. Anim. Sci.* 22:82–85
26. Charlwood, P. A. 1979. The relative affinity of transferrin and albumin for zinc. *Biochim. Biophys. Acta* 581: 260–65
27. Cheng, Y. L. 1932. Hepato-lenticular degeneration (pseudosclerosis, progressive lenticular degeneration and torsion-spasm). Review of literature and report of two cases. *Clin. Med. J.* 46:347–69
28. Cherian, M. G., Goyer, R. A. 1978. Metallothionein and their role in the metabolism and toxicity of metal. *Life Sci.* 23:1–10
29. Chesters, J. K. 1978. Biochemical functions of zinc in animals. *World Rev. Nutr. Diet.* 32:135–64
30. Chesters, J. K., Will, M. 1981. Measurement of zinc flux through plasma in normal and endotoxin-stressed pigs and the effects of Zn supplementation during stress. *Br. J. Nutr.* 46:119–30
31. Chesters, J. K., Will, M. 1981. Zinc transport proteins in plasma. *Br. J. Nutr.* 46:111–18
32. Cousins, R. J. 1976. Zinc thionein and heritable diseases associated with aberrant zinc metabolism. *Lancet* 2:686–87
33. Cousins, R. J. 1979. Regulatory aspects of zinc metabolism in liver and intestine. *Nutr. Rev.* 37:97–103
34. Cousins, R. J. 1979. Regulation of zinc absorption: Role of intracellular ligands. *Am. J. Clin. Nutr.* 32:339–45
35. Cousins, R. J. 1982. Relationship of metallothionein synthesis and degradation to intracellular zinc metabolism. See Ref. 7, pp. 251–60
36. Cousins, R. J. 1982. Mechanism of zinc absorption. In *Clinical, Biochemical and Nutritional Aspects of Trace Elements*, ed. A. S. Prasad, pp. 117–28. NY: Liss
37. Cousins, R. J. 1983. Metallothionein aspects related to copper and zinc metabolism. *J. Inher. Metab. Dis.* 6: In press
38. Cousins, R. J., Smith, K. T., Failla, M. L., Markowitz, L. A. 1978. Origin of low molecular weight zinc-binding complexes from rat intestine. *Life Sci.* 23:1819–26

39. Cousins, R. J., Smith, K. T. 1980. Zinc-binding properties of bovine and human milk in vitro: influence of changes in zinc content. *Am. J. Clin. Nutr.* 33:1083–87
40. Cousins, R. J., Squibb, K. S., Feldman, S. L., de Bari, A., Silbon, B. L. 1977. Biomedical responses of rats to chronic exposure to dietary cadmium fed in ad libitum and equalized regimes. *J. Toxicol. Environ. Health* 2:929–43
41. Cousins, R. J., Weiner, A. L. 1982. Hormonal regulation of copper and zinc metabolism in isolated rat liver parenchymal cells. See Ref. 18, pp. 147–57
42. Cox, R. P., Ruckenstein, A. 1971. Studies on the mechanism of hormonal stimulation of zinc uptake in human cell cultures: hormone-cell interactions and characteristics of zinc accumulation. *J. Cell. Physiol.* 77:71–81
43. Curzon, G., O'Reilly, S. 1960. A coupled iron-caeruloplasmin oxidation system. *Biochem. Biophys. Res. Commun.* 2:284–86
44. Cymbaluk, N. F., Schryver, H. F., Hintz, H. F., Smith, D. F., Lowe, J. E. 1981. Influence of dietary molybdenum in copper metabolism in ponies. *J. Nutr.* 111:96–106
45. Danielson, K. G., Ohi, S., Huang, P. C. 1982. Immunochemical detection of metallothionein in specific epithelial cells of rat organs. *Proc. Natl. Acad. Sci. USA* 79:2301–4
46. Danks, D. M., Stevens, B. J., Campbell, P. E., Gillespie, J. M., Walker-Smith, J., Bloomfield, J., Turner, B. 1972. Menkes' kinky hair syndrome. *Lancet* 1:1100–2
47. Davis, P. N., Norris, L. C., Kratzer, F. H. 1962. Interference of soybean proteins with utilization of trace minerals. *J. Nutr.* 77:217–23
48. DiSilvestro, R. A., Harris, E. D. 1981. A postabsorption effect on L-ascorbic acid on copper metabolism in chicks. *J. Nutr.* 111:1964–68
49. DiSilvestro, R. A., Harris, E. D. 1983. Evaluation of (+)-catechin action on lysyl oxidase activity. *Biochem. Pharmacol.* 32:343–46
50. Dixon, J. W., Sarkar, B. 1974. Isolation, amino acid sequence and copper (II)-binding properties of peptide (1–24) of dog serum albumin. *J. Biol. Chem.* 249:5872–77
51. Dixon, M., Webb, E. C. 1979. *Enzymes.* NY: Academic. 1116 pp.
52. Dreosti, I. E., Tas, S. H., Hurley, L. S. 1968. Plasma zinc and leukocyte changes in weanling and pregnant rats during zinc deficiency. *Proc. Soc. Exp. Biol. Med.* 128:169–74
53. Durnam, D., Palmiter, R. 1981. Transcriptional regulation of the mouse metallothionein-I gene by heavy metals. *J. Biol. Chem.* 256:5712–16
54. Engel, R. W., Price, N. O., Miller, R. F. 1967. Copper, manganese, cobalt, molybdenum balance in preadolescent girls. *J. Nutr.* 92:197–204
55. Epstein, O., Sherlock, S. 1981. Is Wilson's disease caused by a controller gene mutation resulting in perpetuation of the fetal mode of copper metabolism into childhood? *Lancet* 1:303–5
56. Etzel, K. R., Shapiro, S. G., Cousins, R. J. 1979. Regulation of liver metallothionein and plasma zinc by the glucocorticoid dexamethasone. *Biochem. Biophys. Res. Commun.* 89:1120–26
57. Etzel, K. R., Cousins, R. J. 1981. Hormonal regulation of liver metallothionein zinc: independent and synergistic action of glucagon and glucocorticoids. *Proc. Soc. Exp. Biol. Med.* 167:233–36
58. Evans, G. W., Dubois, R. S., Hambridge, K. M. 1973. Wilson's disease: identification of an abnormal copper-binding protein. *Science* 181: 1175–76
59. Evans, G. W., Winter, T. W. 1975. Zinc transport by transferrin in rat portal blood plasma. *Biochem. Biophys. Res. Commun.* 66:1218–24
60. Evans, G. W., Johnson, P. E. 1980. Characterization and quantitation of a zinc-binding ligand in human milk. *Pediatr. Res.* 14:876–80
61. Evans, G. W., Johnson, E. C. 1980. Zinc absorption in rats fed a low-protein diet and a low-protein diet supplemented with tryptophan or picolinic acid. *J. Nutr.* 110:1076–80
62. Evans, G. W., Grace, C. I., Votava, H. J. 1975. A proposed mechanism for zinc absorption in the rat. *Am. J. Phys.* 228:501–5
63. Failla, M. L., Cousins, R. J. 1978. Zinc uptake by isolated rat liver parenchymal cells. *Biochim Biophys. Acta* 538:435–44
64. Failla, M. L., Cousins, R. J. 1978. Zinc accumulation and metabolism in primary cultures of rat liver cells: regulation by glucocorticoids. *Biochim. Biophys. Acta* 543:293–304
65. Failla, M. L., Kiser, R. A. 1983. Hepatic and renal metabolism of copper and zinc in the diabetic rat. *Am. J. Physiol.* 244: 115–121
66. Falchuk, K. H. 1977. Effect of acute disease and ACTH on serum zinc proteins. *N. Engl. J. Med.* 296:1129–34

67. Feldman, S. L., Cousins, R. J. 1976. Degradation of hepatic zinc-thionein following parenteral zinc administration. *Biochem. J.* 160:583–88
68. Feldman, S. L., Squibb, K. S., Cousins, R. J. 1978. Degradation of cadmium thionein in rat liver and kidney. *J. Toxicol. Environ. Health* 4:805–13
69. Feldman, S. L., Failla, M. L., Cousins, R. J. 1978. Degradation of liver metallothioneins in vitro. *Biochim. Biophys. Acta* 544:638–46
70. Fernandez-Pol, J. A. 1978. Morphological changes induced by picolinic acid in cultured mammalian cells. *Exp. Mol. Pathol.* 29:348–57
71. Flagstad, T. 1976. Lethal trait A 46 in cattle. *Nord. Veterinaermed.* 28: 160–69
72. Flagstad, T. 1981. Zinc absorption in cattle with a dietary picolinic acid supplement. *J. Nutr.* 111:1996–99
73. Forbes, R. M. 1961. Excretory patterns and bone deposition of zinc, calcium and magnesium in the rat as influenced by zinc deficiency, EDTA and lactose. *J. Nutr.* 74:194–200
74. Foulkes, E. C., ed. 1982. See Ref. 7
75. Frieden, E., Hsieh, H. S. 1976. Ceruloplasmin: the copper transport protein with essential oxidase activity. *Adv. Enzymol.* 44:187–236
76. Fushimi, H., Hamison, C. R., Ravin, H. A. 1971. Two new copper proteins from human brain. Isolation and properties. *J. Biochem.* 69:1041–54
77. Garvey, J. S., Chang, C. C. 1981. Detection of circulating metallothionein in rats injected with zinc or cadmium. *Science* 211:805–7
78. Ghadimi, H., Pecora, P. 1963. Free amino acids of different kinds of milk. *Am. J. Clin. Nutr.* 13:75–81
79. Giroux, E., Prakash, N. J. 1977. Influence of zinc-ligand mixtures on serum zinc levels in rats. *J. Pharm. Sci.* 66: 391–92
80. Goka, T. J., Stevenson, R. E., Hefferan, P. M., Howell, R. R. 1976. Menkes' disease: a biochemical abnormality in cultured human fibroblasts. *Proc. Natl. Acad. Sci. USA* 73:604–6
81. Goldfischer, S., Sternlieb, I. 1968. Changes in the distribution of hepatic copper in relation to the progression of Wilson's disease (hepatolenticular degeneration). *Am. J. Pathol.* 53:883–901
82. Gollan, J. L. 1975. Studies on the nature of complexes formed by copper with human alimentary secretions and their influence on copper absorption in the rat. *Clin. Sci. and Mol. Med.* 49:237–45
83. Gollan, J. L., Deller, D. J. 1973. Studies on the nature and excretion of biliary copper in man. *Clin. Sci.* 44:9–15
84. Greger, J. L., Snedeker, S. M. 1980. Effect of dietary protein and phosphorous levels on the utilization of zinc, copper and manganese. *J. Nutr.* 110:2243–53
85. Gregoriadis, G., Morell, A. G., Sternlieb, I., Scheinberg, H. 1970. Catabolism of desialylated ceruloplasmin in the liver. *J. Biol. Chem.* 245:5833–37
86. Gubler, C. J., Lahey, M. E., Cartwright, G. E., Wintrobe, M. M. 1953. Studies of copper metabolism. IX. The transport of copper in the blood. *J. Clin. Invest.* 32: 405–14
87. Hahn, C., Evans, G. W. 1973. Identification of a low molecular weight ^{65}Zn complex in rat intestine. *Proc. Soc. Exp. Biol. Med.* 144:793–95
88. Hall, A. C., Young, B. W., Bremner, I. 1979. Intestinal metallothionein and the mutual antagonism between copper and zinc in the rat. *J. Inorg. Biochem.* 11: 57–66
89. Harris, D. I. M., Sass-Kortsak, A. 1967. The infuence of amino acids on copper uptake by rat liver slices. *J. Clin. Invest.* 46:659–77
90. Harris, E. D. 1976. Copper-induced activation of aortic lysyl oxidase in vivo. *Proc. Natl. Acad. Sci. USA* 73: 371–74
91. Harris, E. D., Blount, J. E., Leach, R. M. J. 1980. Localization of lysyl oxidase in hen oviduct: implications in egg shell membrane formation and composition. *Science* 208:55–56
92. Harris, E. D., DiSilvestro, R. A. 1981. Correlation of lysyl oxidase activation with the p-phenylenediamine oxidase activity (ceruloplasmin) in serum. *Proc. Soc. Exp. Biol. Med.* 166:528–31
93. Harris, E. D., Rayton, J. K., DeGroot, J. E. 1977. A critical role for copper in aortic elastin structure and synthesis. In *Elastin and Elastic Tissue,* ed. L. B. Sandberg, W. R. Gray, C. Franzblau, pp. 543–59. NY: Plenum
94. Harris, E. D., Stevens, M., DiSilvestro, R. A. 1983. *Biochemistry*. Submitted for publication
95. Hart, E. B., Steenbock, H., Waddell, J., Elvehjem, C. A. 1928. Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. *J. Biol. Chem.* 77:797–812
96. Held, D. D., Hoekstra, W. G. 1979. *In vitro* degradation of metallothionein by rat liver lysosomes. *Fed. Proc.* 734:2674 (Abstr.)
97. Henkin, R. I. 1974. Metal-albumin-amino acid interactions: chemical and

physiological relationships. In *Protein-Metal Interactions,* ed. M. Friedman, pp. 299–328. NY: Plenum
98. Hill, C. H., Starcher, B. 1965. Effect of reducing agents on copper deficiency in the chick. *J. Nutr.* 85:271–74
99. Holt, C. 1981. Zinc binding ligands in milk: both arguments err seriously. *J. Nutr.* 111:2240–42
100. Horn, N., Jensen, O. A. 1980. Menkes' syndrome: subcellular distribution of copper determined by an ultrastructural histochemical techique. *Ultrastructural Pathol.* 1:237–42
101. Hsieh, H. S., Frieden, E. 1975. Evidence for ceruloplasmin as a copper transport protein. *Biochem. Biophys. Res. Commun.* 67:1326–31
102. Hurley, L. S., Keen, C. L., Young, H. M., Lonnerdal, B. 1982. Effect of chelate of zinc concentration in rat maternal and pup tissues. *Fed. Proc.* 41:2982 (Abstr.)
103. Ishimura, Y., Hayaishi, O. 1973. Noninvolvement of copper in the L-tryptophan 2,3-dioxygenase reaction. *J. Biol. Chem.* 248:8610–12
104. Jackson, M. J., Jones, D. A., Edwards, R. H. T. 1981. Zinc absorption in the rat. *Br. J. Nutr.* 46:15–27
105. Jamison, M. H., Sharma, H., Case, R. M., Braganza, J. M. 1981. Pancreatic secretions assist bile in limiting copper absorption in the rat. *Gut* 22:A866–67
106. Kagi, J. H., Nordberg, M. 1979. *Metallothionein.* Basel: Birkhauser. 378 pp.
107. Karin, M., Herschman, H. R. 1979. Dexamethasone stimulation of metallothionein synthesis in HeLa cell cultures. *Science* 204:176–77
108. Keen, C. L., Saltman, P., Hurley, L. 1980. Copper nitrilotriacetate: a potent therapeutic agent in the treatment of a genetic disorder of copper metabolism. *Am. J. Clin. Nutr.* 33:1789–1800
109. Kelsay, J. L., Jacob, R. A., Prathers, E. S. 1979. Effect of fiber from fruits and vegetables on metabolic responses of human subjects. III. Zinc, copper and phosphorous balances. *Am. J. Clin. Nutr.* 32:2307–11
110. Kirchgessner, M., Grassmann, E. 1970. The dynamics of copper absorption. In *Trace Element Metabolism in Animals,* ed. C. F. Mills, pp. 277–86. Edinburg: Livingstone
111. Kirchgessner, M., Roth, H. P. 1980. Biochemical changes of hormones and metalloenzymes in zinc deficiency. In *Zinc in the Environment - Part II: Health Effects,* ed. J. O. Nriagu, pp. 71–103. NY: Wiley
112. Kirchgessner, M., Roth, H. P., Weigand, E. 1976. Biochemical changes in zinc deficiency. In *Trace Elements in Human Health and Disease,* ed. A. S. Prasad, O. Oberleas, pp. 189–225. NY: Academic
113. Kowarski, S., Blair-Stanek, C. S., Schachter, D. 1974. Active transport of zinc and identification of zinc-binding protein in rat jejunal mucosa. *Am. J. Physiol.* 226:401–7
114. Krieger, I., Evans, G. W. 1980. Acrodermatitis enteropathica without hypozincemia: therapeutic effect of a pancreatic enzyme preparation due to a zinc-binding ligand. *Pediatrics* 96:32–35
115. LaBadie, G. U., Beratis, N. G., Price, P. M., Hirschhorn, K. 1981. Studies of the copper-binding proteins in Menkes' and normal cultured skin fibroblasts lysates. *J. Cell. Physiol.* 106:173–78
116. Lau, A. L., Failla, M. L., 1983. Urinary excretion of zinc, copper and iron in the streptazotocin diabetic rat. *Am. J. Physiol.* Submitted for publication
117. Leucke, R. W., Olman, M. E., Baltzer, B. V. 1968. Zinc deficiency in the rat: effect on serum and intestinal alkaline phosphatase activities. *J. Nutr.* 94: 344–50
118. Linder, M. C., Moore, J. R. 1977. Plasma ceruloplasmin. Evidence for its presence in and uptake by heart and other organs of the rat. *Biochim. Biophys. Acta* 499:329–36
119. Lombeck, I., Schnippering, H. G., Ritzl, F., Feinendegen, L. E., Bremer, H. J. 1975. Absorption of zinc in acrodermatitis enteropathica. *Lancet* 1:855
120. Lonnerdal, B., Stanislowski, A. G., Hurley, L. S. 1980. Isolation of a low molecular weight zinc binding ligand from human milk. *J. Inorg. Biochem.* 12:71–78
121. Lonnerdal, B., Keen, C. L., Sloan, M. V., Hurley, L. S. 1980. Molecular localization of zinc in rat milk and neonatal intestine. *J. Nutr.* 110:2414–19
122. Lonnerdal, B., Schneeman, B. O., Keen, C. L., Hurley, L. S. 1980. Molecular distribution of zinc in biliary and pancreatic secretions. *Biol. Trace Elem. Res.* 2:149–58
123. Lovstad, R. A. 1982. The protective action of ceruloplasmin on copper ion stimulated lysis of rat erythrocytes. *Int. J. Biochem.* 14:585–89
124. Mann, J. R., Camakaris, J., Danks, D. 1979. Copper metabolism in mottled mouse mutants. *Biochem. J.* 180:613–19

125. Marceau, N., Aspin, N., Sass-Kortsak, A. 1970. Absorption of copper 64 from gastrointestinal tract of the rat. *Am. J. Physiol.* 218:377–83
126. Marceau, N., Aspin, N. 1972. Distribution of ceruloplasmin-bound ^{67}Cu in the rat. *Am. J. Physiol.* 222:106–10
127. Marceau, N., Aspin, N. 1973. The intracellular distribution of the radiocopper derived from ceruloplasmin and from albumin. *Biochim. Biophys. Acta* 293: 388–50
128. Martell, A. E. 1960. The relationship of chemical structure to metal binding action. In *Metal-Binding in Medicine*, ed. M. J. Seven, L. A. Johnson. Philadelphia, PA: Lippincott. 350 pp.
129. Martin, M. T., Licklider, K. F., Brushmiller, J. G., Jacobs, F. A. 1981. Detection of low-molecular-weight copper (II) and zinc (II) interactions following ethambutol administration. *Agents Actions* 11:296–305
130. May, P. M., Smith, G. L., Williams, D. R. 1982. Computer calculation of zinc (II)-complex distribution in milk. *J. Nutr.* 112:1990–93
131. Mayo, K. E., Palmiter, R. D. 1982. Glucocorticoid regulation of the mouse metallothionein I gene is selectively lost following amplification of the gene. *J. Biol. Chem.* 257:3061–67
132. McCall, J. T., Davis, G. K. 1961. Effect of dietary protein and zinc on the absorption and liver deposition of radioactive and total copper. *J. Nutr.* 74:45–50
133. McCormick, C. C., Menard, M. P., Cousins, R. J. 1981. Induction of hepatic metallothionein by feeding zinc to rats of depleted zinc status. *Am. J. Physiol.* 240:E414–21
134. McMaster, D., Steel, L., Love, A. H. G. 1982. Zinc absorption by vascularly perfused small intestine. In *Trace Element Metabolism in Man and Animals-4*, pp. 121–24. Berlin: Springer-Verlag
135. Menard, M. P., Cousins, R. J. 1983. Zinc transport by brush border membrane vesicles from rat intestine. *J. Nutrition.* Submitted for publication
136. Menard, P., McCormick, C. C., Cousins, R. J. 1981. Regulation of intestinal metallothionein biosynthesis in rats by dietary zinc. *J. Nutr.* 111:1353–61
137. Menard, M. P., Oestreicher, P., Cousins, R. J. 1983. Zinc transport by isolated, vascularly perfused rat intestine and brush border vesicles. In *Nutritional Bioavailability of Zinc*, ed. G. C. Inglett, pp. 233–46. Washington DC: Am. Chem. Soc.
138. Mercer, J. F. B., Lazdins, I., Stevenson, T., Carnokaris, J., Danks, D. M. 1981. Copper induction of translatable metallothionein messenger RNA. *Biosci. Rep.* 1:793–800
139. Methfessel, A. H., Spencer, H. 1974. Intestinal absorption and secretion of ^{65}Zn in the rat. In *Trace Element Metabolism in Animals-2*, ed. W. G. Hoekstra, J. W. Suttie, H. E. Ganther, W. Mertz, 541–43. Baltimore, MD: University Park Press
140. Mills, C. F. 1956. Studies of the copper compounds in aqueous extracts of herbage. *Biochem. J.* 63:187–90
141. Mondovi, B., Rotilio, G., Finazzi, A., Scioscia-Santoro, A. 1964. Purification of pig-kidney diamine oxidase and its identity with histaminase. *Biochem. J.* 91:408–15
142. Morell, A. G., Shapiro, J. R., Scheinberg, I. H. 1961. Copper-binding proteins of human liver. In *Wilson's Disease, Some Current Concepts*, ed. J. M. Walshe, J. N. Cumings, pp. 36–42. Oxford: Blackwell
143. Morgan, W. T. 1981. Interactions of the histidine-rich glycoprotein of serum with metals. *Biochemistry* 20:1054–61
144. Moynahan, E. J. 1974. Acrodermatitis enteropathica: a lethal inherited human zinc deficiency disorder. *Lancet* 2:399–400
145. National Research Council, Subcommittee on Zinc. 1979. *Zinc*, ed. R. I. Henkin, et al. Baltimore, MD: University Park Press. 471 pp.
146. Oberleas, D., Muhrer, M. E., O'Dell, B. L. 1966. The availability of zinc from foodstuffs. In *Zinc Metabolism*, ed. A. S. Prasad, pp. 225–38. Springfield, IL: Thomas
147. Oestreicher, P., Cousins, R. J. 1982. Influence of intraluminal constituents on zinc absorption by isolated, vascularly perfused rat intestine. *J. Nutr.* 112:1978–82
148. Oh, S. H., Deagan, J. T., Whanger, P. D., Weswig, P. H. 1978. Biological function of metallothionein. V. Its induction in rats by various stresses. *Am. J. Physiol.* 234:E282–85
149. Otvos, J. D., Armitage, I. M. 1980. Structure of the metal clusters in rabbit liver metallothionein. *Proc. Natl. Acad. Sci. USA* 77:7094–98
150. Owen, C. A. Jr. 1965. Metabolism of radiocopper (Cu^{64}) in the rat. *Am. J. Physiol.* 209:900–4
151. Owen, C. A. Jr. 1982. *Biochemical*

Aspects of Copper. Park Ridge, NJ: Noyes.
152. Parisi, A. F., Vallee, B. L. 1970. Isolation of a zinc macroglobulin from human serum. *Biochemistry* 9:2421–26
153. Pecoud, A., Donzel, P., Schelling, J. L. 1975. Effect of foodstuffs on the absorption of zinc sulfate. *Clin. Pharmacol. Ther.* 17:469–74
154. Pedrosa, F. O., Pontremoli, S., Horecker, B. L. 1977. Binding of Zn^{2+} to rat liver fructose-1,6 bisphosphatase and its effect on the catalytic properties. *Proc. Natl. Acad. Sci. USA* 74:2742–45
155. Pekarek, R. S., Evans, G. W. 1975. Effect of acute infection and endotoxemia on zinc absorption in the rat. *Proc. Soc. Exp. Biol. Med.* 150:755–58
156. Pekas, J. C. 1971. Pancreatic incorporation of ^{65}Zn and histidine ^{14}C into secreted proteins of the pig. *Am. J. Physiol.* 220:799–803
157. Pekas, J. C. 1966. Zinc-65 metabolism: gastrointestinal secretion by the pig. *Am. J. Physiol.* 211:407–13
158. Pickart, L. 1981. The use of glycylhistidyllysine in culture systems. *In Vitro* 17:459–66
159. Pickart, L., Thaler, M. M. 1980. Growth-modulating tripeptide (glycylhistidyllysine): association with copper and iron in plasma, and stimulation of adhesiveness and growth of hepatoma cells in culture by tripeptide-metal ion complexes. *J. Cell. Physiol.* 102:129–39
160. Planas, J., Frieden, E. 1973. Serum iron and ferroxidase activity in normal, copper-deficient, and estrogenized rooster. *J. Physiol.* 225:423–28
161. Prasad, A. S., Oberleas, D. 1970. Binding of zinc to amino acids and serum proteins *in vitro*. *J. Lab. Clin. Med.* 76: 416–25
162. Prohaska, J. R. 1981. Comparison between dietary and genetic copper deficiency in mice: copper-dependent anemia. *Nutr. Res.* 1:159–67
163. Prohaska, J. R., Lukasewycz, O. A. 1981. Copper deficiency suppresses the immune response of mice. *Science* 213:559–61
164. Pulido, P., Kagi, J. H. R., Valle, B. L. 1966. Isolation and some properties of human metallothionein. *Biochemistry* 5: 1768–77
165. Rayton, J. K., Harris, E. D. 1979. Induction of lysyl oxidase with copper Properties of an in vitro system. *J. Biol. Chem.* 254:621–26
166. Reed, D. W., Passon, P. G., Hultquist, D. E. 1970. Purification and properties of pink copper protein from human erythrocytes. *J. Biol. Chem.* 245:2954–61
167. Richards, M. P., Cousins, R. J. 1975. Mammalian zinc homeostasis: requirements for RNA and metallothionein synthesis. *Biochem. Biophys. Res. Commun.* 64:1215–23
168. Richards, M. P., Cousins, R. J. 1976. Metallothionein and its relationship to the metabolism of dietary zinc in rats. *J. Nutr.* 106:1591–99
169. Richards, M. P., Cousins, R. J. 1976. Zinc-binding protein: Relationship to short term changes in zinc metabolism. *Proc. Soc. Exp. Biol. Med.* 153:52–56
170. Riordan, J. R., Jolicoeur-Paquet, L. 1982. Metallothionein accumulation may account for intracellular copper retention in Menkes' disease. *J. Biol. Chem.* 257:4639–45
171. Roeser, H. P., Lee, G. R., Nacht, S., Cartwright, G. E. 1970. The role of ceruloplasmin in iron metabolism. *J. Clin. Invest.* 49:2408–17
172. Royce, P. M., Camakaris, J., Danks, D. M. 1980. Reduced lysyl oxidase activity in skin fibroblasts from patients with Menkes' syndrome. *Biochem. J.* 192: 579–86
173. Ryden, L., Deutsch, H. F. 1978. Preparation and properties of the major binding component in human fetal liver, its identification as metallothionein. *J. Biol. Chem.* 253:519–24
174. Saylor, W. W., Morrow, F. D., Leach, M. Jr. 1980. Copper and zinc binding proteins in sheep liver and intestine: effects of dietary levels of the metals. *J. Nutr.* 110:460–68
175. Schricker, B. R., Forbes, R. M. 1978. Studies on the chemical nature of a low molecular weight zinc binding ligand in rat intestine. *Nutr. Rep. Int.* 18: 159–66
176. Sharoyan, S. G., Shaljian, A. A., Nalbandyan, R. M., Buniatian, H. C. 1977. Two copper-containing proteins from white and gray matter of brain. *Biochim. Biophys. Acta* 493:478–87
177. Schultze, M. O., Elvehjem, C. A., Hart, E. B. 1936. Further studies on the availability of copper from various sources as a supplement to iron in hemoglobin formation. *J. Biol. Chem.* 115:453–57
178. Shapiro, S. G., Cousins, R. J. 1980. Induction of rat liver metallothionein mRNA and its distribution between free and membrane-bound polysomes. *Biochem. J.* 190:755–64
179. Smith, K. T., Failla, M. L., Cousins, R. J. 1979. Identification of albumin as the plasma carrier for zinc absorption by perfused rat intestine. *Biochem. J.* 184: 627–33

180. Smith, K. T., Cousins, R. J. 1980. Quantitative aspects of zinc absorption by isolated, vascularly perfused rat intestine. *J. Nutr.* 110:316–23
181. Soboscinski, P., Canterbury, W. J., Mapes, C. A., Dinterman, R. E. 1978. Involvement of hepatic metallothionein in hypozincemia associated with bacterial infection. *Am. J. Physiol.* 234: E399–406
182. Solomons, N. W., Cousins, R. J. 1983. Zinc. In *Absorption and Malabsorption of Mineral Nutrients,* ed. I. H. Rosenberg, N. W. Solomons. NY: Liss. In Press
183. Song, M. K., Adham, N. F. 1978. Role of prostaglandin E_2 in zinc absorption in the rat. *Am. J. Physiol.* 234:E99–105
184. Sourkes, T. L. 1968. Properties of the monoamine oxidase of rat liver mitochondria. *Adv. Pharmacol.* 6A:61–69
185. Starcher, B. C., Glauber, J. G., Madaras, J. G. 1980. Zinc absorption and its relationship to intestinal metallothionein. *J. Nutr.* 110:1391–97
186. Starcher, B., Hill, C. H. 1965. Hormonal induction of ceruloplasmin in chicken serum. *Comp. Biochem. Physiol.* 15: 429–34
187. Sternlieb, I. 1980. Copper and liver. *Gastroenterology* 78:1615–28
188. Sternlieb, I., Morell, G., Tucker, W. D., Greene, M. W., Scheinberg, I. H. 1961. The incorporation of copper into ceruloplasmin in vivo: studies with copper^{64} and copper^{67}. *J. Clin. Invest.* 40:1834–40
189. Sternlieb, I., van den Hames, C. J. A., Morell, A. G., Alport, S., Gregariadis, G., Scheinbert, I. H. 1973. Lysosomal defect of hepatic copper excretion in Wilson's disease (hepatolenticular degeneration). *Gastroenterology* 64:99–103
190. Steyn-Parve, E. P., Beinert, H. 1958. On the mechanism of dehydrogenation of fatty acyl derivatives of coenzyme A. *J. Biol. Chem.* 233:853–61
191. Sullivan, J. F., Williams, R. V., Wisecarver, J., Etzel, K., Jetton, M. M., Magee, D. F. 1981. The zinc content of bile and pancreatic juice in zinc-deficient swine. *Proc. Soc. Exp. Biol. Med.* 166: 39–43
192. Suso, F. A., Edwards, H. M. Jr. 1971. Ethylenediaminetetraacetic acid and ^{65}Zn binding by intestinal digesta, intestinal mucosa and blood plasma. *Proc. Soc. Exp. Biol. Med.* 138:157–62
193. Suso, F. A., Edwards, H. M. 1972. Binding of EDTA, histidine and acetylsalicylic acid to zinc-protein complex in intestinal content, intestinal mucosa and blood plasma. *Nature* 236:230–32
194. Suttle, N. F. 1974. Recent studies of the copper-molybdenum antagonism. *Proc. Nutr. Soc.* 33:299–305
195. Swerdel, M. R., Cousins, R. J. 1982. Induction of kidney metallothionein and metallothionein messenger RNA by zinc and cadmium. *J. Nutr.* 112:801–9
196. Tejwani, G. A., Pedrosa, F. O., Pontremoli, S., Horecker, B. L. 1976. Dual role of Zn^{2+} as inhibitor and activator of fructose 1,6-bisphosphatase of rat liver. *Proc. Natl. Acad. Sci. USA* 73:2692–95
197. Underwood, E. J. 1977. Zinc. In *Trace Elements in Human and Animal Nutrition,* pp. 196–242. NY: Academic
198. Underwood, E. J. 1977. Copper. See Ref. 197, pp. 56–108
199. Unger, R. H., Orci, L. 1981. Glucagon and the A cell. *N. Engl. J. Med.* 304: 1518–24
200. Van Campen, D., Gross, E. 1968. Influence of ascorbic acid on the absorption of copper by rats. *J. Nutr.* 95:617–22
201. Van Campen, D. R., Kowalski, T. J. 1971. Studies on zinc absorption ^{65}Zn binding by homogenates of rat intestinal mucosa. *Proc. Soc. Exp. Biol. Med.* 136:294–97
202. Van Campen, D. R., House, W. A. 1974. Effect of a low protein diet on retention of an oral dose of ^{65}Zn and on tissue concentrations of zinc, iron, and copper in rats. *J. Nutr.* 104:84–90
203. Victery, W., Levenson, R., Vander, A. J. 1981. Effect of glucagon on zinc excretion in anesthetized dogs. *Am. J. Physiol.* 240:F299–305
204. Victery, W., Smith, J. M., Vander, A. J. 1981. Renal tubular handling of zinc in the dog. *Am. J. Physiol.* 241:F532–39
205. Vohra, P., Kratzer, F. H. 1966. Influence of various phosphates and other complexing agents on the availability of zinc for turkey poults. *J. Nutr.* 89: 106–12
206. Vohra, P., Krantz, E., Kratzer, F. H. 1965. Formation constants of certain zinc-complexes by ion-exchange method. *Proc. Soc. Exp. Biol. Med.* 121:422–25
207. Walshe, J. M. 1981. The discovery of the therapeutic use of d-penacillamine. *J. Rheumatol.* 8(Suppl.7):3–8
208. Weigand, E., Kirchgessner, M. 1978. Homeostatic adjustments in zinc digestion to widely varying dietary zinc intake. *Nutr. Metab.* 22:101–12
209. Weiner, A. L., Cousins, R. J. 1980. Copper accumulation and metabolism in primary monolayer cultures of rat liver

parenchymal cells. *Biochim. Biophys. Acta* 629:113–25

210. Weiner, A. L., Cousins, R. J. 1983. Hormonal produced changes in ceruloplasmin synthesis and secretion in primary cultured rat hepatocytes-relationship to hepatic copper metabolism. *Biochem. J.* 212:297–304
211. Whitehouse, M. W., Walker, R. W. 1978. Copper and inflammation. *Agents Actions* 8:85–90
212. Yunice, A. A., King, R. W. Jr., Kraikitpanitch, S., Haygood, C. C., Lindemann, R. D. 1978. Urinary zinc excretion following infusions of zinc sulfate, cysteine, histidine, or glycine. *Am. J. Physiol.* 235:F40–45