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Copper and zinc metabolism in health and disease: speciation and interactions

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It has long been evident that the metabolism of trace elements cannot be considered in isolation. A wide range of nutritional and physiological factors can influence their uptake, transport and storage, with subsequent enhancement of susceptibility to deficiency or toxicity states. Interactions occur with other trace elements and these can be classified as competitive or non-competitive, direct or indirect. However, their physiological or toxicological significance is sometimes debatable, partly because they were demonstrated under extreme experimental conditions where massive doses of the antagonistic metal were administered, often by parenteral routes and to animals whose trace element status was already severely compromised. Our understanding of the mechanisms of the interactions is frequently limited and indeed in only a few cases has it been possible to describe these on a molecular basis.

It is almost traditional to cite the early work of Hill & Matrone (1970) as the first serious attempt to describe trace element interactions on a rational basis. They postulated that elements with similar physical or chemical properties will act antagonistically to each other biologically. The implication was that such metals could compete for binding sites on transport proteins or on enzymes requiring metals as co-factors. Over subsequent years, much evidence was produced in support of this view but rarely was the specific site of interaction identified, other than at tissue level. In part this reflected somewhat embarrassing limitations in our knowledge of the proteins involved in the intracellular and extracellular transport and storage of trace metals.

COPPER–ZINC INTERACTIONS

The mutual antagonism between Zn and Cu has been regarded as a prime example of competitive biological interactions between metals with similar chemical and physical properties. This has been demonstrated in many investigations in a range of species. Thus, excessive Zn supply has been shown to inhibit intestinal absorption, hepatic accumulation and placental transfer of Cu, as well as to induce clinical and biochemical signs of Cu deficiency (Bremner *et al.* 1976; Hall *et al.* 1979; L'Abbe & Fischer, 1984; Yardick *et al.* 1989). The situation regarding the reverse interaction, namely the effects of Cu on Zn metabolism, has been less clear. Excessive Cu supply can affect hepatic Zn metabolism in some species but there is no consistent evidence that Zn absorption is seriously affected (Hall *et al.* 1979; Blalock *et al.* 1988). This casts some doubt on the mutuality of the interaction. Most of the early investigations were largely descriptive and they did little to elucidate the precise molecular basis or locus of the interaction.

Cu and Zn occupy a similar part of the periodic table, the Zn^{2+} and Cu^{+} ions being isoelectronic and having similar ionic radii. Both metals readily form chelates with O-, S-, or N-containing ligands but not always with the same stereochemical arrangement of bonds. However, unlike Zn which occurs in only one valency state, Cu can occur in two, as Cu^{+} and Cu^{2+}; indeed there have been suggestions that Cu^{3+} may also have a

transient existence. Cu, therefore, can play a major role in redox reactions and participates via the Haber-Weiss reaction in the generation of O free-radicals which promote cellular instability. Zn, on the other hand, has a stabilizing function and reputedly protects cell membranes against lipid peroxidation, and sulphydryl groups against oxidation (Willson, 1989). It plays an important role in maintaining the conformation of proteins, and 'Zn fingers' constitute ubiquitous structural elements in many transcription factors (Falchuk, 1993).

COPPER AND ZINC ENZYMES

There are differences also between Cu and Zn in the types of enzymes with which they are associated. Thus, Cu is frequently present in oxidases where its ability to exist in two oxidation states is integral to the activity of the enzyme. Zn is a component of dehydrogenases, proteases and esterases, its role often being to maintain the conformation of the enzyme, possibly as a β sheet or in crosslinking between S atoms (Williams, 1989). To the best of our knowledge, there is no evidence of substitution of Cu by Zn, or *vice versa*, in any of these enzymes. One enzyme does contain both metals, superoxide dismutase (*EC* 1.15.1.1), but at clearly-defined sites, with Zn again having a structural role and Cu occurring at the active site and exercising a redox function. Williams (1989) has commented on the complementary role of Zn and Cu enzymes in regulating the composition of extracellular matrix and, thereby, cell growth and multiplication. Thus, Zn is a component of the collagenases and proteases that break down the extracellular matrix and, thus, permit cell movement, whereas Cu functions in the crosslinking and strengthening of the collagen which is an integral component of the matrix. The proper balance between Zn and Cu may be an essential ingredient of the alternating demands of stability and growth of the soft structures of an organism. Although changes in the activity of Cu enzymes may occur in animals or cells exposed to high levels of Zn, there is no evidence that Cu is displaced from enzymes by Zn, rather it seems that the availability of Cu has been reduced.

TRANSPORT IN PLASMA

If metalloenzymes do not constitute a site of interaction between Cu and Zn, does the competition occur at the level of their transport systems? The enhanced uptake of Zn, Cd, Pb, Mn and other metals in Fe-deficient animals is believed to reflect such competition and the ability of the metals to use transport systems for Fe, possibly including transferrin (Hamilton *et al.* 1978). However, none of the plasma proteins which bind Cu or Zn appear to be involved in their mutual interaction. Caeruloplasmin and transcuprein bind only Cu whereas α2-macroglobulin binds only Zn. Both metals bind to albumin, which is involved in their transport from the intestine to the liver but at different sites on the protein, the Cu being bound specifically at an N-terminal histidine residue. Moreover, the molar concentration of albumin in plasma far exceeds that of Cu and Zn, making it unlikely that competitive displacement of Cu by Zn, or *vice versa* would ever occur. Small amounts of plasma Cu and Zn may be bound to metallothionein, and there is evidence of Cu displacing Zn from the protein *in vivo* (Bremner *et al.* 1987). This could result in increased renal uptake and urinary excretion of Cu as Cu–metallothionein.

INBORN ERRORS OF COPPER METABOLISM

Further indication that the metabolism of Cu and Zn is subject to quite different control is provided by the occurrence of metal-specific inborn errors of metabolism. Thus, Menkes' disease and Wilson's disease in humans and related conditions in animals such as Bedlington terriers, mottled mice, toxic milk mice and Long-Evans Cinnamon (LEC) rats are associated with severe disturbances in Cu metabolism, with only minor changes in tissue Zn concentration and never any clinical signs of Zn deficiency or toxicity. The basic defect in Cu metabolism in Wilson's disease and LEC rats is excessive hepatic accumulation of Cu and consequent liver damage. Plasma caeruloplasmin levels and biliary Cu excretion are reduced, implying a defect in the mechanisms whereby Cu is excreted from the liver (Gibbs & Walshe, 1980; Sugawara *et al.* 1993). In contrast, Menkes' disease and the mottled mouse syndrome represent severe and often fatal forms of Cu deficiency, characterized by reductions in Cu absorption, liver Cu content and the activities of Cu-dependent enzymes (Danks, 1989). However, Cu concentrations in tissues such as the gut and kidneys increase, as they do also in cultured fibroblasts, indicating that there are defects in Cu transport systems in some tissues. Zn metabolism is essentially normal in Menkes' disease, Wilson's disease and related diseases.

Until recently, little was known of the proteins involved in the control of Cu transport or of the genetic and metabolic basis of Wilson's disease or Menkes' disease. However, the successful cloning of the Menkes' gene (Mercer *et al.* 1993) and the gene for the murine homologue (Mercer *et al.* 1994) has transformed our views on the regulation of Cu transport. As yet, the Menkes' gene product has not been isolated but its structure has been deduced through sequence data of a cDNA. The gene product appears to consist of 1500 amino acids with six extended runs of hydrophobic amino acids typical of the transmembrane domains of a membrane-spanning protein. It contains five or six repeats of a metal-binding sequence motif at the N-terminal part of the protein which is similar in alignment to bacterial Cd and Hg resistant systems in which P-type ATPases regulate heavy-metal efflux. Another single sequence at residues 1044–1050 contains an aspartic acid residue that serves as a phosphorylation site for an ATP-driven cation pump. This is believed to drive metal ions from the cytosol, in a similar manner to the K^+–Na^+ ATPase (*EC* 3.6.1.37) which regulates intracellular Na concentrations. The Menkes' gene is expressed in tissues such as the gut, heart, brain, lung, muscle, pancreas and placenta but significantly not in the liver (Vulpe *et al.* 1993). Absence of the Cu transporter ATPase would be expected to result in impaired Cu efflux from cells, as indeed is found in Menkes' disease.

Similar studies on the structure of the Wilson's disease gene indicate that this also encodes a Cu-transporting ATPase (Bull *et al.* 1993). The gene shows high identity with the Menkes' gene, homology being 50% overall and 79% in the transduction, channel, phosphorylation and ATP-binding regions. Significantly the Wilson's disease gene is expressed mainly in the liver and kidney, which is appropriate for the clinical manifestations of the disease. This contrasts with the occurrence of the Menkes' gene in most tissues other than the liver and kidney (Vulpe *et al.* 1993). It is interesting that the Wilson's disease gene contains cysXXcys motifs in each of its metal-binding regions, together with one cysXcys motif in the transduction region. Similar motifs are characteristic of many transition-metal-binding proteins, are abundant in metallothionein and bind Cu in the reduced state. It is possible that this ability to reduce Cu is integral to the

incorporation of Cu into apocaeruloplasmin and to the excretion of Cu in bile, both of which processes are impaired in Wilson's disease.

The discovery of these genes for putative Cu-transporting ATPases with contrasting tissue distribution is providing new insights into the study of Cu transport in health and disease. The occurrence of metal transporters with high degrees of homology in bacteria, animals and man indicates that they are of fundamental importance and also that further forms of the protein may await discovery. Although the gene products of the Cu-transporting genes have yet to be isolated and characterized, there is no indication that they are implicated in the transport of Zn or constitute the site of interaction between these metals.

CYSTEINE-RICH INTESTINAL PROTEIN (CRIP)

Genetic disturbances in Zn metabolism occur in acrodermatitis enteropathica in humans and in A46 Friesian cattle, a defect in Zn absorption resulting in a severe Zn deficiency. The specific cause of the impaired Zn absorption is unknown but there have been suggestions that a CRIP plays a role in the transport of Zn across the gut mucosal epithelium in normal animals (Hempe & Cousins, 1991, 1992). CRIP gene expression is most pronounced in the villus cells of the small intestine, reaching a peak of transcription during weaning in the rat (Birkenmeier & Gordon, 1986). The deduced amino acid sequence for CRIP shows some similarity to that of certain ferredoxins (Birkenmeier & Gordon, 1986) and contains a metal-binding LIM motif ($C\text{-}X_2\text{-}C\text{-}X_{17\text{-}19}\text{-}H\text{-}X_2\text{-}C\text{-}X_2\text{-}C\text{-}X_2\text{-}C\text{-}X_{7\text{-}11}\text{-}(C)\text{-}X_8\text{-}C$) which is also present as a two-copy tandem in 'Zn-finger' proteins (Wang *et al.* 1992; Levenson *et al.* 1993; Kosa *et al.* 1994).

In support of its proposed role as an intracellular Zn-transport molecule, CRIP binds a large proportion of the Zn entering the intestinal mucosal cells at low lumen Zn concentrations and binding is saturable at higher levels. Nevertheless, expression of CRIP does not appear to be Zn-dependent and there are no nucleic acid sequences upstream of the CRIP gene which have high homology to known metal regulatory elements (Levenson *et al.* 1994). Furthermore, the 1,25-hydroxycholecalciferol-stimulated increase in Zn transport across a monolayer of Caco-2 cells grown on permeable filter supports was accompanied by a reduction in CRIP expression (Fleet *et al.* 1993). Thus, the role of CRIP in Zn transport remains unresolved.

Studies of metal binding *in vitro* to proteins with LIM motifs, including CRIP, demonstrate the binding of Cu^+ with a maximal stoichiometry of three per motif compared with two for Zn^{2+} (Kosa *et al.* 1994). The tertiary folding of the Zn and Cu proteins are consequently distinct and this would probably have significant effects on their function. However, there is no evidence for binding of metals other than Zn to these proteins *in vivo*.

STRESS-INDUCED DISTURBANCES IN COPPER AND ZINC METABOLISM

Differences in Cu and Zn metabolism are also evident in response to physical and inflammatory stress and infection. These effects are mediated by a range of gluco-corticoids and cytokines, including interleukins-1 and -6. A characteristic response is a sustained increase in plasma Cu concentrations, which is associated almost entirely with caeruloplasmin; this is widely recognized as an acute-phase protein. That the primary

response is induction of caeruloplasmin synthesis in the liver and not Cu mobilization was confirmed by the demonstration that injection of Cu-deficient rats with interleukin-1 resulted in production of caeruloplasmin-protein, although it was devoid of enzyme activity because of the absence of the necessary Cu co-factor (Barber & Cousins, 1988). In contrast, the characteristic change in Zn metabolism after stress is a rapid and fairly transient decrease in plasma Zn concentrations which is associated with an increase in liver Zn content (Cousins, 1989). That these changes are directly related is supported by the results of compartmental analysis in rats injected with Zn^{65} following treatment with endotoxin or butyryl cyclic AMP (Dunn & Cousins, 1989; Lowe *et al.* 1991). The clearance of Zn^{65} from the plasma could be described by a two-compartment model in which the initial pool (Q_A) represented mainly plasma and the second pool (Q_B) was located primarily within the liver. Although plasma Zn concentrations following endotoxin treatment fell, both Q_A and Q_B increased. However, the increase in Q_A may have resulted from adverse effects of the endotoxin on cellular permeability. It appeared that Q_B represented part of a metabolic pool within the liver and that this increased substantially in response to both the Zn loading and endotoxin treatment in a manner similar to that of the protein metallothionein. This is consistent with a plethora of reports that glucocorticoids, cytokines such as the interleukins and tumour necrosis factor, and other stress factors can induce metallothionein synthesis principally in the liver but also in other tissues (Bremner, 1987). Indeed, it appears that the decrease in plasma Zn levels after stress or infection is a direct consequence of the sequestration by the newly-induced thionein of Zn from the circulation. The physiological significance of these changes in Cu and Zn metabolism are still the subject of conjecture. There could be some advantage to the host in reducing circulating levels of Zn or possibly the increased production of caeruloplasmin and metallothionein is designed to cope with the oxidant challenge induced by the stress, since both these proteins can act as free radical scavengers.

METALLOTHIONEIN

Metallothionein is a low-molecular-weight cysteine-rich metal-binding protein which has a family of isoforms in some species and occurs in most tissues. Its synthesis is induced by metals such as Zn, Cu and Cd and also by a range of pathophysiological factors which promote gene transcription directly in the case of metals, or indirectly through changes in cytokine or steroid levels (Palmiter, 1987). Transcription, which is elevated 1–2 h after metal-loading, involves activation of a nuclear-binding factor by metals and its binding to metal-responsive elements (MRE) situated upstream of the metallothionein gene. Recently, a transcription factor, MTF-1, which activates the mouse metallothionein promoter and contains six 'Zn fingers' has been cloned (Radtke *et al.* 1993). The process of factor activation by metals is as yet unknown but Palmiter (1994) has proposed a mechanism involving an inhibitor protein which normally interacts with the 'Zn fingers' of MTF-1 thereby preventing its binding to MRE. In the presence of Zn it is proposed that the inhibitor dissociates from MTF-1 and promotion of transcription can proceed. Transcription studies of BHK cells transfected with a metallothionein MRE-β-galactosidase (*EC* 3.2.1.23) gene construct and also an MTF-1 construct with a viral promoter sequence showed that the expression of β-galactosidase by Cu was highly dependent on the presence of Zn in the incubation medium, whereas Cd induction did not appear to be Zn-dependent (Palmiter, 1994). The relative induction of metallo-

thionein gene transcription by Cu and Zn is species and tissue or cell dependent and is influenced by the way in which the metal is administered. For example, metallothionein mRNA levels were significantly elevated in the kidney but not the small intestine of rats fed on a high-Cu diet (36 mg Cu/kg) whereas increased levels of message were detected in both tissues following high dietary Zn treatment (180 mg Zn/kg) (Blalock *et al.* 1988). The different isoforms of metallothionein code from distinct genes which are linked on the same chromosome and are usually coordinately expressed (Lehman-McKeeman *et al.* 1991). However, differential expression of isoform genes has been noted in certain species and in response to different inducers. For example, sheep have at least four functioning metallothionein genes (Peterson *et al.* 1988) and when comparing the isoform induction by Cu and Zn in sheep fibroblast cultures, Cu appears to be a less effective inducer of MT-1c transcription (Peterson & Mercer, 1988). Furthermore, there is growing evidence for discrepancies in the translational control of isoform expression (Lehman-McKeeman *et al.* 1988; Paynter *et al.* 1990) and possible differences in the functional roles of metallothionein isoforms has not been adequately addressed.

The cysteine residues in metallothionein are highly conserved between isoforms, their distinctive characteristics arising from substitution of 2–25% of the remaining amino acids. The metal-saturated form of metallothionein contains seven divalent metals such as Zn and Cd but Cu, which is always present in the Cu^+ form, has a binding ratio of 12 *in vivo*. All the cysteine residues are involved in metal binding in two separate domains or metal–S clusters. Cysteine:metal values are 3:1 for Zn metallothionein but only 2:1 for Cu–metallothionein and yet the arrangement of S atoms around each metal is tetrahedral and trigonal respectively. This implies that there are bridging S atoms between some of the metal atoms. In addition to these differences in the stereochemical arrangement of ligands around the Cu and Zn atoms, the metals differ in their preferential binding for the two domains within the protein. On titration of the apoprotein with Zn or Cd, the C-terminal α domain fills preferentially with four metal atoms followed by three in the N-terminal β domain (Nielson & Winge, 1983; Stillman & Zelazowski, 1988). Cu on the other hand shows preferential binding to the β domain (Nielson & Winge, 1984). The avidity with which these metals bind to metallothionein decreases in the order Cu>Cd>Zn and, in contrast to apoprotein, the binding of Cd to Zn_7–metallothionein is initially random, although redistribution of metals to the more thermodynamically stable and domain-specific position can be activated by raising the temperature to 65° (Stillman *et al.* 1987). Therefore, in the presence of Cd or Cu, Zn could be regarded as having a passive binding role to complete the full complement of metals and, thus, maintain the structural integrity of the protein.

Concentrations of metallothionein in tissues depend on their Zn and to a lesser extent Cu concentrations, which is consistent with the inducibility of protein synthesis by these metals. Almost invariably, metallothionein binds most of the hepatic Zn above a threshold concentration which is similar to that found in Zn-deficient animals. However, the binding of hepatic Cu to metallothionein is more variable and depends on factors such as species, route of Cu administration, and Zn status. For example, compared with many species in which hepatic metallothionein levels are largely unaffected by moderately elevated levels of dietary Cu, dogs and pigs show increased levels of this protein. A factor common to these two species is the absence of a specific Cu-binding site on serum albumin (SA) and there is evidence that when rat primary hepatocyte cultures are incubated with Cu bound to various SA, cellular metallothionein but not cellular Cu

is higher in the presence of canine SA (Beattie *et al.* 1993). Although metallothionein often contains Zn as the only bound metal, it is only in extreme Cu-loading situations that Cu is present without Zn as a secondary bound metal. Cu–metallothionein is generally absent from the livers of Zn-deficient animals, even at very high liver Cu concentrations, despite the fact that Cu can induce synthesis of metallothionein in these animals. This may reflect in part the reduced biological half-life of metallothionein in Zn-deficient animals. Although metallothionein is generally regarded as a cytosolic protein, it has been shown by immunocytochemical techniques to occur within the nucleus. The distribution of the protein between these two compartments is variable, with concentrated nuclear metallothionein appearing during cell proliferation, for example at S-phase during epidermal growth factor-stimulated hepatocyte proliferation (Tsujikawa *et al.* 1991), during liver regeneration after hepatectomy (Tohyama *et al.* 1993) and in liver during fetal development (Nartey *et al.* 1987). Aggregated forms of the protein also accumulate in particulate fractions of the liver, including the lysosomes, after extreme Cu loading (Bremner, 1987).

The rapid changes in metallothionein concentrations in response to metal administration imply that it has a regulatory function, the nature of which is not yet entirely clear. One possibility is that the protein acts as a Zn buffer, designed to prevent increased intracellular concentrations of free Zn ions (Bremner, 1993). This provides a mechanism whereby Zn can be transiently stored before it is incorporated into metalloenzymes, used to support growth and development, or excreted. There is also good evidence that the protein is involved in the cellular detoxification of Cu. This is particularly evident in cell lines in which the level of metallothionein expression has been enhanced, as these cells are much more tolerant of Cu than are the wild-type cells (Freedman, 1989). The protective effect of Zn against Cu-induced liver damage, also, has been ascribed to an increased binding of Cu to metallothionein. Similarly, species which are able to accumulate large amounts of hepatic Cu as metallothionein are generally tolerant of Cu-induced liver dysfunction. However, fulminant hepatitis occurs at lower liver Cu concentrations in LEC rats than in normal strains, despite the fact that metallothionein concentrations are much greater in the former animals (Sugawara *et al.* 1992).

There have been many attempts to ascribe tissue-specific functions to metallothionein, despite the fact that its ubiquity implies a general role in control of cellular metabolism. For example, it was suggested many years ago that metallothionein is involved in the control of intestinal Zn absorption (Richards & Cousins, 1976). This was based on the observation of an inverse relationship between the efficiency of absorption and mucosal metallothionein concentrations, which suggested that binding of dietary Zn to metallothionein on the mucosa provided a mechanism for restricting basolateral transfer of Zn to the circulation. In essence, metallothionein could act as a mucosal block to Zn absorption. Attractive though this hypothesis is, the mucosal metallothionein content does not change significantly over a physiological range of dietary Zn intakes, where the efficiency of absorption varies as part of the homeostatic control mechanism (Hall *et al.* 1979; Blalock *et al.* 1988). Moreover, the amount of Zn trapped on mucosal metallothionein represents only a small proportion of the dietary Zn intake. It seems, therefore, that the increase in mucosal metallothionein concentrations that occurs at high dietary Zn intakes represent a partial failure rather than an essential component of homeostatic control mechanism, in so far as it may only occur after mucosal Zn concentrations have

increased. Indeed, the affinity of metallothionein for Zn is of such magnitude that at high dietary Zn levels, a large proportion of the metal bound to the putative intracellular transporter CRIP is scavenged by metallothionein. The evidence that metallothionein plays a role in the control of Cu absorption is even less convincing. No changes in mucosal metallothionein concentrations occur in response to substantial changes in Cu supply (Hall *et al.* 1979; Blalock *et al.* 1988). Although the decreased efficiency of Cu absorption in brindled mice is associated with excessive accumulation of Cu bound to intestinal metallothionein, this is almost certainly a consequence of the defect in the efflux of Cu from the mucosal cells.

Despite this, intestinal metallothionein may be implicated in the antagonistic effect of Zn on Cu metabolism. Thus, high dietary Zn intakes inhibited the absorption of oral Cu^{64} in rats, this being associated with an increase in the binding of the Cu^{64} in the mucosa in the form of metallothionein (Hall *et al.* 1979; Oestreicher & Cousins, 1985). A dose–response trial revealed that the absorption of Cu was only inhibited at Zn intakes that caused substantial increases in mucosal metallothionein concentration. Moreover, the amount of Cu^{64} trapped in the mucosa as metallothionein was almost equivalent to the Zn-induced reduction in the carcass accumulation of the isotope. It seems, therefore, that high Zn intakes result in increased production in the gut of metallothionein which then binds Cu in preference to Zn, thereby blocking transfer of the Cu across the basolateral membrane and inhibiting absorption. This hypothesis assumes that Cu on metallothionein is not available for reabsorption after desquamation of the intestinal cells. This does not necessarily represent the only mechanism whereby Zn affects Cu accumulation, as it seems that low levels of Zn can affect Cu metabolism without inducing mucosal metallothionein production (Oestreicher & Cousins, 1985). Moreover, there are direct effects of Zn on hepatic Cu distribution that appear to be modulated by metallothionein (Bremner, 1993).

CLINICAL IMPLICATIONS

The interaction between Cu and Zn is of importance in both animal production and clinical medicine. Thus, Zn supplements have been found to induce hypocupraemia and associated neutropenia and microcytosis when used to treat patients with sickle cell anaemia; these conditions were corrected by Cu administration (Prasad *et al.* 1978). Moreover, they have induced negative Cu balance (Sandstead *et al.* 1982) and reduced erythrocyte superoxide dismutase activities (Fischer *et al.* 1984) in normal subjects. This suggests that care should be taken in the use of Zn supplements, particularly where Cu status is suboptimal.

However, Zn supplementation offers a means of treating conditions associated with excessive Cu accumulation, whether caused by dietary intake or genetic disturbances in Cu metabolism. Thus, Zn supplements reduce the incidence of Cu toxicosis in sheep (Bremner *et al.* 1976) and are now used to treat Wilson's disease in humans (Hoogenraad *et al.* 1978; Brewer *et al.* 1983). Brewer *et al.* (1993) have established the optimum-dosage regimen to reduce Cu absorption and to restore Cu balance. It appears that a daily dose of 75 mg Zn as zinc acetate, divided into at least two doses and given between meals, is most effective (Brewer *et al.* 1993). This treatment can be used as maintenance therapy and for treatment of presymptomatic patients. That the Zn-induced decrease in Cu absorption is due to increased production of intestinal metallothionein is suggested by

the results of direct measurement of the protein in intestinal biopsies (Yuzbasiyan-Gurkan *et al.* 1992). Intestinal metallothionein concentrations were linearly correlated with urinary Zn levels which reflected Zn status. However, it is possible that Zn also affects the hepatic distribution of Cu and increases the proportion bound to metallothionein, with concomitant reduction in its hepatotoxicity.

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