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COPPER DEFICIENCY IN HUMANS

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CONTENTS

Copper (Cu) was established as an essential nutrient in the 1920s and 1930s by laboratory studies in rats and by field observations in sheep and cattle. Most of the features seen in these and other species can now be explained by the biological roles of Cu that have been recognized over the subsequent years. Knowledge of Cu requirements, absorption, and transport and of the biological roles of Cu has accumulated over the past 60 years, but remains frustratingly incomplete today. Despite extensive research in many species, it is still not possible to relate all the effects of deficiency to specific Cu functions, nor are all the subtle effects of long-term marginal deficiency known. Much remains to be learned about the transport of Cu to and within body cells. A number of extensive reviews have been published and can be consulted for background not covered in this review (10, 13, 18, 41, 58, 81, 96, 102). Well-established facts, if referenced at all, are supported by refer-

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ence to latest review publications rather than by citing first descriptions or all relevant papers.

DOES COPPER DEFICIENCY OCCUR IN HUMANS?

It was more difficult to establish the effects of Cu deficiency in humans than in animals, and debate about its existence as a clinical problem waxed during the 1950s and 1960s (12, 103). In 1972, the discovery of a genetic disease (Menkes disease) with features explicable by Cu deficiency finally settled the matter (14, 19, 20). This "experiment of nature" provided more decisive evidence than other studies in humans over the preceding decades. At about the same time, strong evidence came from two other powerful approaches that had been used deliberately in the laboratory, but were applied accidentally in man. Elemental diets given under strictly controlled conditions had been used in rats to establish the essential requirement for trace elements and ultratrace elements. Total parenteral nutrition (TPN) in humans restricted intake to highly purified components and unintentionally produced clear-cut evidence of deficiencies of Cu (24, 44) and of other trace elements. Competitive inhibitors have been valuable tools in experimental biochemistry, physiology, and pharmacology. The administration of large doses of Zn to patients with sickle cell anemia (68) accidentally caused Cu deficiency by specifically interfering with intestinal absorption of Cu.

There is no longer any debate about the essential role of Cu in humans and the main effects of severe deficiency are well established, even though not all these effects can be explained adequately.

IS MILD CHRONIC COPPER DEFICIENCY A SIGNIFICANT NUTRITIONAL PROBLEM IN HUMANS?

This is the important practical issue for the future. The effects, if any, may vary at different stages of life and some individuals may be at greater risk than others. A more detailed knowledge of Cu functions, requirements, and transport is needed before these issues can be resolved.

Before addressing this interesting question, it is necessary to review some of the established facts about Cu and human health. Some background information on the effects of Cu deficiency in animals is also important.

BIOLOGICAL ROLES OF COPPER

The current understanding of the roles of Cu is based on the functions of the known Cu enzymes (10), plus copper's firmly established role in the disulfide bonding of keratin by an unknown mechanism (Table 1) (29). Cu is found in

Table 1 Copper enzymes in humans

Common name (Ref.)	EC number	Functional role	Known or *possible* consequence of deficiency
Cytochrome C oxidase (10)	1.9.3.1	Electron-transport chain	*Muscle weakness; cardiomyopathy; brain degeneration*
Superoxide dismutase (10)	1.15.1.1	Free radical detoxification	*Membrane damage; other free radical damage*
Tyrosinase (10) (monophenol monooxygenase)	1.14.18.1	Melanin production	Failure of pigmentation
Dopamine-β-hydroxylase (10, 50) (dopamine-β-monoxygenase)	1.14.17.1	Catecholamine production	*Neurological effects, type uncertain*
Lysyl oxidase (10, 63)	1.4.3.13	Cross-linking of collagen and elastin	Vascular rupture; loose skin and joints; osteoporosis; emphysema; bladder diverticulae
Ceruloplasmin (10, 31)	1.16.3.1	Ferroxidase, amine oxidase, Cu transport	*Anemia; deficient supply of Cu to other tissues*
Clotting factor V (53)		Blood clotting	*Bleeding tendency*
Enzyme not known (29)		Cross-linking of keratin (disulfide bonds)	Pili torti

the active site of each of the enzymes. The roles of some of these enzymes are well known, but there is uncertainty about others. Additional copper atoms are present in many of the enzymes. Ceruloplasmin is an enzyme capable of oxidizing ferrous iron and a variety of amines, but it is still not certain whether these functions are relevant in vivo (28). Its role in copper transport is still debated (13). Coagulation factor V is a recent addition to the list of Cu proteins (enzymes) (53). The close relationship of the amino acid and DNA sequences of factor VIII, factor V, and ceruloplasmin (53) has led to speculation regarding a role for Cu in factor VIII.

Most of the features of severe Cu deficiency can be explained by failure of one or more of the Cu enzymes. For instance, depigmentation is easily explained by tyrosinase deficiency, and lysyl oxidase deficiency is responsible for the defects in elastin and collagen that underlie the connective tissue abnormalities. Some effects like anemia and brain damage are not so easily explained (see below).

The importance of reductions in the activity of cytochrome oxidase in causing the manifestations of Cu deficiency has probably been underestimated. Many authors have suggested that the reduction to 50% of normal activity seen in Cu deficiency is unlikely to cause disease (e.g. 85). It is now clear that genetic defects of cytochrome oxidase can cause serious neurological, cardiac, and muscle disease even when the activity of the enzyme is reduced to only about 50% of normal (as measured in vitro) (21).

COPPER HOMEOSTASIS AND THE TRANSPORT OF COPPER

Maintaining a steady level of copper in the body of a healthy subject depends primarily upon a balance between intestinal absorption and biliary excretion, with small additional losses in sweat and by the desquamation of skin. Biliary excretion is capable of increasing substantially when there is copper overload. The conservation of copper by the kidney is also important. In attempting to give a more detailed account, one soon exhausts firmly established knowledge. The brief notes that follow draw upon data regarding humans, rats, mice, and other species. More extensive reviews are available (13, 41, 58, 96). Essentially the processes of copper transport have to supply an adequate amount of Cu to the Cu enzymes without allowing Cu to exert the toxic effects of which it is capable as it passes to and through cells. The very short life of ^{64}Cu has hampered studies of its incorporation into Cu enzymes and has rendered impossible any investigation of the fate of the Cu released when these enzymes are broken down. Although ^{67}Cu has a somewhat longer half-life, it has been available from very few suppliers and at high cost.

^{64}Cu administered orally is absorbed rapidly, with peak absorption at 30

minutes, and 30% of the dose is absorbed by 8 hours in rats (56) and in humans (88). Absorption is maximal in the upper small intestine with both active and passive components to the system, which is little influenced by the form in which copper in presented (55).

Within the intestinal mucosal cells Cu can interact with metallothionein. It is not clear whether metallothionein plays any role in the normal absorptive process or whether it can prevent excessive absorption when the intake of Cu is high. Metallothionein is involved in the important mechanism by which excessive zinc (Zn) intake can block Cu absorption (32). Zinc is a stronger inducer of metallothionein production than Cu, yet Cu can displace Zn from metallothionein (5). Large doses of Zn can trap Cu in the intestinal mucosal cells bound to metallothionein until the mucosal cells are shed. The dose of Zn required is well above normal intake, but the margin seems to be narrower in humans, especially in premature infants. This mechanism is exploited in the Zn therapy of Wilson disease, in which the dose required (50 mg before each meal) is approximately five times the nutritional requirement (39, 40). Zinc administered for other purposes can cause Cu deficiency (68).

Egress of Cu from the intestinal mucosal cells into the portal bloodstream is not understood, but ^{64}Cu appears rapidly in the peripheral bloodstream bound to albumin. Preferential accumulation in the liver occurs in humans (99), mice, and rats (52, 55, 63), but there is also substantial uptake by other tissues within the first few hours after administration. Most review articles assume that Cu-albumin is the transport form in the portal blood, but this has not been shown directly. In vitro hepatocytes do not accept Cu directly from albumin, and Cu histidine serves as an intermediary (25, 36, 59, 64). It is possible that most of the direct transfer from intestine to liver is as Cu-histidine and that Cu-albumin acts as a transient store of Cu in the bloodstream. Cu-albumin may actually slow the transfer to the liver, not hasten it (47, 64).

Even less is known about the distribution of Cu to tissues other than the liver. The conventional view has been that Cu is taken up by the liver, incorporated into ceruloplasmin, and carried by it to other tissues. However, this process cannot explain the high proportion of orally administered ^{64}Cu found in nonhepatic tissues in the first hours after oral administration (52). In vivo studies have shown that the carrier to which Cu is attached (albumin or ceruloplasmin) influences the relative rates of uptake by different tissues (55, 63). Most of the numerous in vitro studies of cultured cells have not been very helpful in understanding this process, because most have used unphysiological media and because most workers have assumed that the system giving the most rapid rate of uptake is likely to be the physiological one. In fact, it is very likely that the reverse is true and that transport systems in vivo supply Cu to cells in a regulated rather than rapid fashion.

The mechanism by which Cu eventually enters the bile and is excreted is

very poorly understood. There is not even consensus about the molecular form of Cu present in the bile (77).

Although the loss of Cu in the urine is insignificant, the role of the normal kidney in Cu homeostasis is not. It is likely that substantial amounts of Cu are filtered through the glomerulus and reabsorbed in the tubules. Heavy losses of Cu are observed in patients who are loaded intravenously with individual amino acids or with mixtures of amino acids (38, 94), as well as in patients with Menkes disease and in brindled mice, in which tubular reabsorption of Cu is defective (7, 19, 20).

Ceruloplasmin is an important Cu protein. It is a large glycoprotein containing six atoms of Cu per molecule, has an oxidase activity against many substrates (10, 13, 31), and contributes approximately 90% of the Cu present in plasma. Consequently, measurement of plasma Cu is effectively measurement of ceruloplasmin. It can be measured immunologically or by its oxidase activity. Its function is still not defined. It has a ferroxidase activity that has been considered important in the release of iron from stores and amine oxidase activity against the range of biologically active amines. It may also have a role in Cu transport, especially to red blood cells (63), heart muscle (49), and aortic endothelium (89).

Metallothioneins are also important in Cu homeostasis. They are unusual proteins that contain 61 amino acids, 20 of which are cysteine. Mice and rats have two metallothioneins; humans and sheep have more than five metallothioneins (5, 13, 34). They clearly play a major role in relation to Zn, which is not further discussed here. Many of the findings suggest that metallothioneins act mainly to bind and detoxify excess copper, especially in liver and kidney (5, 13, 34). However, some studies have shown ^{67}Cu to bind the metallothionein as soon as it is taken into liver cells (91); this suggests a role in transport within the liver cells.

It is apparent that many further Cu ligands are necessary to explain the safe passage of Cu through cells, between cells, to sites of Cu enzyme synthesis, and finally into the bile for excretion. The existence and role of transcuprein (100) needs confirmation, and further study on neurocuprein (57) would be welcome.

COPPER DEFICIENCY IN ANIMALS

The effects of Cu deficiency observed in those species that can be manipulated experimentally form an important background to understanding human Cu deficiency. Copper deficiency has been observed in the field in most farm species, and has been studied extensively in sheep, cattle, pigs, and poultry (96). Rats have been tested most extensively in the laboratory, with fewer studies on mice, guinea pigs, and rabbits. Interest in mice has in-

tensified since the discovery that the mottled mutants are Cu deficient (see below).

Combining all these studies it is possible to list a wide range of features of Cu deficiency (Table 2), most of which fit with predictions based upon the known Cu functions (Table 1). Species differences are quite marked and the age at which an animal experiences the deficiency influences the effects. For instance, osteoporosis, anemia, and "steely wool" are prominent in sheep, but vascular disease does not occur; arterial rupture is a major problem in pigs (8) and poultry (62). Neurological effects are seen in lambs or mice deprived of Cu in utero (72, 96), but not in lambs subjected to Cu deficiency after birth or in other species.

Anemia and neutropenia are seen in all animal species and in nutritional Cu deficiency in humans. Lack of the ferroxidase activity of ceruloplasmin and consequent failure of release of iron from tissue stores has been blamed for the anemia (28), but this is unlikely to be the complete explanation (70, 71). Patients with Wilson disease or Menkes disease may have very little ceruloplasmin, yet they do not develop anemia or neutropenia (16) nor do brindled mice (7, 71). Severe reduction of cytochrome oxidase and superoxide dismutase has been shown in bone marrow of Cu-deficient mice, but not in brindled mice (71). This might explain both the anemia and the neutropenia. Reduction of erythropoietin has been described in rats (105).

Osteoporosis is another feature seen in all species. It is probably the consequence of inadequate cross-linking of collagen caused by lysyl oxidase deficiency. Lysyl oxidase deficiency also leaves elastin inadequately cross-linked (and therefore more soluble than normal when tissues are studied in the laboratory), a defect that is responsible for the aortic rupture and emphysema seen in several species (8, 35, 62).

Myocardial disease due to Cu deficiency was first noted as a cause of sudden death ("falling disease") in cattle in Western Australia in the late 1930s and has also been seen in sheep (96). Myocardium is generally hypertrophied, but may become dilated and thin, and may even rupture. Fibrosis is found diffusely through the hypertrophied muscle with a distribution quite different from that seen in ischemic heart disease seen in humans. The coronary arteries have not been described as abnormal in the naturally occurring disease in cattle and sheep, nor in Cu deficiency in rats. Indeed, coronary artery resistance is decreased in affected rat hearts (73). A functional defect in myocardial contractibility was demonstrated in isolated perfused hearts from Cu-deficient rats in a study that also showed diminished catecholamine levels in the myocardium (73). The collagen framework of the myocardium has been shown to be abnormal (6) and the ECG abnormalities have been described (98). The type of myocardial disease seen in these various animals seems to have much more in common with the cardiomyopathy seen in cytochrome

Table 2 Some effects of copper deficiency in different species

	Nutritional deficiency in					Mutants			
						Mouse		Human	
Feature	Sheep	Cattle	Pig	Rat/mouse	Human	Mo^{br}	Mo^{blo}	Menkes	Occipital horn
Anemia	+	+	+	+	+	–	–	–	–
Neutropenia	+	+	+	+	+	–	–	–	–
Abnormal hair	++	+	NR[a]	+	NR	+	+	+	+/–
Arterial disease	–	–	++	+	NR	–	+	+	NR
Myocardial disease	+	+	+	+	NR	–	–	–	–
Osteoporosis	+	+	+	+	+	+/–	+	+	+
Cerebellar ataxia	+(F)	NR	+(F)	+(F)	NR	+	–	+	–
Other brain damage	+(F)	NR	+(F)	+(F)	NR	+	–	+	+/–

[a] NR = not recorded.

oxidase deficiency in humans (21) than with human ischemic heart disease. Lysyl oxidase deficiency and dopamine-β-hydroxylase deficiency may also be playing a part.

Hypercholesterolemia has been described in several studies of Cu-deficient adult rats (45) but could not be induced by Cu deficiency in young rats or mice (74). Most of the excess cholesterol has been found in high-density lipoproteins (HDL fraction) (48) and a reduction of HDL receptors was observed.

Neurological effects are seen only in some species (sheep, mice, and humans) and only when Cu deficiency commences prenatally. Swayback, or enzooitic ataxia, of lambs is the only naturally occurring form in animals. Neuronal loss is marked in the cerebellum and spinal tracts with extensive demyelination (84, 85, 96). Ataxia with neuronal loss in cerebellum and motor cortex (104) occurs in the brindled mouse mutant (42), which resembles closely human Menkes disease (18). Similar neurological abnormalities have been produced in newborn mice born to Cu-deficient dams (67, 75) but the brain lesions in these animals have not been described. Deficiencies of cytochrome oxidase and of dopamine-β-hydroxylase are probably important in the production of these lesions (72, 75, 85). The existence of a critical phase during which Cu deficiency can cause brain damage is suggested by the results of Cu therapy of brindled mice and of mice born to Cu-deficient dams. Treatment at 7 days is successful, but treatment after 10 days is not (52, 61, 67).

Claims of a specific role of copper in myelination have become entrenched in articles on copper, but there is little evidence of a specific role (72). This claim began because myelin deficiency is so marked in swayback, but this condition has the features of demyelination, not defective synthesis, and the distribution of lesions corresponds with the distribution of neuronal loss (72, 83–85). The same is true in prenatal Cu deficiency in rats. In this species prenatal deficiency must be very severe to produce any changes in the brain (75, 106).

The mottled (Mo) series of X-chromosomal mutants in mice have defects in Cu transport that resemble very closely those seen in Menkes disease (17, 18, 42). The various mouse mutants are generally regarded as allelic, but allelism cannot be distinguished from closely linked gene loci on the available information. These mice were originally noticed because of patchy depigmentation of the coat in females, with lethality or serious abnormalities in males, which are depigmented all over. Males with the mottled (mo) mutation die in utero, as do dappled (Mo^{dp}) males. Brindled (Mo^{br}) males live to 15 days and die of neurological defects; they resemble classical Menkes disease in humans. Tortoise-shell (Mo^{to}) and viable-brindled (Mo^{vbr}) live longer. Blotchy (Mo^{blo}) males have a near normal life span and no neurological effects, but die of arterial rupture and/or emphysema. Brindled mice have been studied in

most detail. Copper is poorly absorbed, but the Cu that is absorbed accumulates attached to metallothionein in kidney and some other tissues. The disease effects are due to Cu enzyme deficiency.

COPPER DEFICIENCY IN HUMAN GENETIC DISEASES

The most extreme form of Cu deficiency is seen in Menkes disease (16, 20), an X-linked condition that is probably homologous to the mottled mutants in the mouse (18). The basic lesion is complex, involving Cu malabsorption, increased urinary loss, and abnormal intracellular Cu transport, which in turn causes abnormal distribution between organs and within cells (16), but it is still reasonable to suppose that all of the effects are in the end due to inadequate supply of the Cu needed for Cu enzyme synthesis and activity. Profound effects are seen in brain, bones, arteries, other connective tissues, hair, pigmentation, and general bodily function (16). The effects begin in utero, slowing growth and damaging many organs before birth. Hypothermia is a problem throughout the early months.

Interference with cross-linking of elastin and collagen can be blamed for many of the features of the disease (16)—premature rupture of the membranes leading to premature birth, lax skin and joints, elongation and dilatation of major arteries leading to rupture and hemorrhage, subintimal thickening with partial occlusion of major arteries, hernias, and diverticulae of bladder and ureters causing recurrent infection or rupture. Osteoporosis, flaring of metaphyses, fractures of metaphyseal edges, and Wormian bones in cranial sutures may all be secondary to collagen abnormalities.

Neurological development rarely progresses beyond the 6–8 week level and even these functions are lost during the ensuing months. Pathologically, the abnormalities of Purkinje cells in the cerebellum are especially severe (43) and ataxia is striking in mild cases (69). The profound deficiency of all neurological function in classical Menkes disease generally obscures the cerebellar component of the disease.

Lack of pigmentation of skin and hair and abnormal spiral twisting (pili torti) and fragility of the hair add to the characteristic appearance of the affected babies (19). Disulfide bonding of keratin is defective as in Cu-deficient sheep (20).

Most of the somatic features can be explained by the known Cu functions (Table 2), but it is not so easy to determine the basis of the neurological defects; deficiencies of cytochrome oxidase, superoxide dismutase, and dopamine-β-hydroxylase are all possible culprits.

Two related genetic conditions have been described: mild Menkes disease (69) and the occipital horn syndrome (46, 66, 80). In both these conditions and in classical Menkes disease, cultured cells accumulate excessive levels of

Cu attached to metallothionein (66) and exhibit lysyl oxidase deficiency (46). In mild Menkes disease, ataxia and mild learning difficulties are seen with a variable degree of connective tissue abnormality (69). Connective tissue defects (hernias, bladder and ureteric diverticulae, loose skin) predominate in the occipital horn syndrome (80) along with the ossified occipital lesion that gives the condition its name.

It would be unwise to conclude simply that lysyl oxidase is more susceptible to Cu deficiency than other Cu enzymes in humans, because the abnormal intracellular distribution of Cu in these mutants may be changing the balance of effects.

Anemia and neutropenia are not seen in these conditions (16) and susceptibility to infection is not increased, relative to other brain-damaged children.

SEVERE NUTRITIONAL COPPER DEFICIENCY IN HUMANS

Copper deficiency has been demonstrated in only a small number of patients reported in the medical literature. Most have been young babies or elderly patients with special medical factors causing the deficiency. Deficiency due to simple inadequacy of Cu intake has been described in many ex-premature babies (1, 2, 82, 90, 102) and in young Peruvian and Chilean children with severe malnutrition and chronic diarrhea (12, 30, 97).

Anemia, neutropenia, and osteoporosis have been the principal features. The anemia can be normocytic or megaloblastic. Bone marrow examination may show megaloblasts and/or sideroblasts and an arrest in neutrophil maturation (2, 24). Even less is known about the mechanism underlying the neutropenia.

Osteoporosis has been observed in most reported cases. Some have shown flaring of the metaphyses and fractures of the margins of the metaphyses, as seen in Menkes disease. Other connective tissue defects like loose skin, loose joints, and arterial abnormalities have not been mentioned nor specifically excluded. Arteriography has not been reported. It is likely that long periods of Cu deficiency are necessary for these features to develop or there may be critical phases during life when Cu deficiency can cause these effects.

The fine hair and depigmentation observed in Peruvian children with Cu deficiency (30) is difficult to interpret because these features are also described in kwashiorkor and the children were grossly malnourished. One cannot tell whether protein-caloric deficiency was causing these effects in the Peruvian children or whether Cu deficiency could be involved in causing these features in many cases of severe malnutrition.

Specific neurological abnormalities have not been described, although

hypotonia has been mentioned in some babies (2). The earlier onset (in utero), greater severity, and longer duration of copper deficiency in Menkes disease may be necessary to cause brain damage and some other defects.

Circumstances Leading to Severe Copper Deficiency

Prematurity is clearly a predisposing factor and is discussed below. Chronic diarrhea may have been important in the Cu deficiency observed in Peruvian children (10, 30). Graham (cited in 10) felt that some interference with absorption or else chronic intestinal loss of Cu must be playing a part in these patients.

Parenteral nutrition has been a frequent cause of Cu deficiency. There have been many reports in babies and in elderly people in the last 15 years (24, 37, 44, 102). These events have provided some of the best available data on the minimum requirement of Cu for human subjects (see below). Inadequate Cu content of the solutions is compounded by increased renal loss when amino acids are infused at rates exceeding renal tubular absorption. (94). Copper deficiency has also been described in children undergoing chronic peritoneal dialysis (3).

Oral administration of Zn has also caused Cu deficiency in humans. The mechanism was discussed above. In some patients, the Zn was given for specific reasons in sickle cell anemia (68) or in delayed wound healing. A current vogue of liberally prescribing Zn supplements (with or without multivitamin preparations) by some practitioners of alternative forms of medicine is a serious concern. The practice often follows analysis of trace element levels in hair and/or fingernails of patients with various symptoms and sufficient money to pay for the procedures. The interpretation of these results is difficult and generally of dubious validity (see below). Some people buy vitamins and Zn across the counter of "health food" shops and consume large quantities in the belief that they are harmless and good for health. This practice should be discouraged.

Diagnosis of Severe Copper Deficiency

The features described thus far can definitely be ascribed to Cu deficiency and are seen in severe Cu deficiency due to identifiable factors. Diagnosis of deficiency of this degree is simple once the possibility is considered. Serum Cu and ceruloplasmin levels are reduced to a degree that leaves no doubt, e.g. to 30% of the normal level. Tissue Cu levels have not been reported in these cases, but one could predict that liver Cu levels would be low. Serum Cu and ceruloplasmin levels return to normal within a few days after Cu therapy is given (54).

MILD CHRONIC COPPER DEFICIENCY IN HUMANS

For every essential nutrient one must anticipate some circumstances that will cause severe deficiency and a particular array of harmful effects. If the effects are serious most affected individuals will have suffered deficiency for only a relatively short time. One must also anticipate that there will be some individuals whose intake is a little below the minimum required over a longer period. It is always hard to identify these individuals, partly because the minimum requirement is difficult to determine and may vary from person to person, and partly because the effects of prolonged mild deficiency may differ qualitatively from those of severe deficiency. All these difficulties have been apparent in the study of Cu nutrition in humans (and, for that matter, in animals).

Since specific nutritional deficiencies are among the most treatable of all human diseases, it is important to determine whether mild chronic Cu deficiency does contribute to human ill health and to establish the nutritional requirement for the metal under various circumstances.

Possible Features of Chronic Copper Deficiency

The known biological effects of Cu and the features observed in severe Cu deficiency provide a list of candidate features. Methods of seeking evidence of Cu deficiency in patients with these candidate features are discussed below.

ANEMIA AND NEUTROPENIA Anemia and neutropenia are features of severe Cu deficiency. The patients identified as Cu deficient have generally suffered anemia refractory to other treatments. If good methods of recognizing mild Cu deficiency can be established, they should be applied to patients with less severe anemia in whom Cu deficiency may be only one factor. Obviously, this should be done in carefully designed studies, not in individual cases. The presence of anemia and neutropenia should be documented in studies of patients with other disorders possibly caused by Cu deficiency.

OSTEOPOROSIS Osteoporosis is a marked feature in most patients with severe Cu deficiency. It must also be considered as a possible consequence of marginal deficiency. Osteoporosis is a very frequent problem in the elderly, especially in females. Although estrogen deficiency is undoubtedly important in the genesis of postmenopausal osteoporosis, it does not seem to explain the whole problem. In a multifactorial problem like osteoporosis, no single etiological factor would be expected in all cases. Rather one would expect one factor to be important in some cases, but less important in others. In evaluating the role of Cu deficiency one should be looking for a subset of cases with demonstrable deficiency and should choose a sample large enough to reveal a

significant subset (e.g. at least 100 cases if 10% is considered a significant subset). If ceruloplasmin does really transport Cu to connective tissues, then the estrogen/Cu interaction may be relevant since serum ceruloplasmin levels are partly determined by estrogen levels (31).

Although Cu deficiency has been demonstrated in a few premature babies with multiple effects, including osteoporosis, the role of less severe Cu deficiency in the very common osteoporosis of very-low-birth-weight babies has not been determined. Follow-up studies of bone density in treated cases of infantile Cu deficiency would also be of interest.

ARTHRITIS Arthritis is another common condition in which Cu status may be relevant. Collagen and elastin are important in cartilage so Cu must be necessary. One might anticipate a role in osteoarthritis rather than in rheumatoid arthritis or other joint disorders of early onset. Copper has been used in arthritis for many years without any scientific basis. Many arthritis sufferers wear Cu bracelets. Advocates of the copper/arthritis connection have pointed out that Cu is absorbed transdermally from metallic Cu and some have advocated dermal application of organic Cu complexes such as Cu aspirinate for the treatment of arthritis (87). The same workers have argued that the beneficial effects of penicillamine in rheumatoid arthritis might be the result of a redistribution of Cu between tissues.

The exaggerated claims of these proponents should not deter others from taking seriously the possibility of a causative association. Careful studies are needed in a large group of patients once good methods of assessing Cu status have been developed.

ARTERIAL DISEASE Arterial disease is another possible Cu-related disorder. The gross arterial disease seen in Menkes disease (19) must mean that humans should be ranked, along with pigs, poultry, rats, and mice, among those species whose arteries are susceptible to the effects of Cu deficiency. The principal abnormality in the arterial walls in Cu deficiency is in elastin, with great increases in soluble elastin and reduction in cross-linked mature fibrils (8, 62). Consequently one might expect the impact of the disorder to be maximal in the aorta and large arteries, with less effect on smaller vessels, which have more smooth muscle in their walls. This is the pattern seen in Menkes disease (19) and in the animals susceptible to the arterial effects of Cu deficiency (8, 62).

In recent years several groups have sought evidence of Cu deficiency in patients with aortic aneurysms, but, unfortunately, the data do not really settle the matter one way or the other (23, 92).

LOSS OF PIGMENTATION Loss of pigmentation might also be expected in chronic Cu deficiency since this is a pronounced features in most species.

Black sheep can be used as reliable indicators of Cu-deficient pastures. Diminished pigmentation of skin and hair are so common in elderly humans that is hard to see how to use this indicator in this group. This feature might be of more use in dark-skinned races or in younger patients.

MYOCARDIAL DISEASE Myocardial disease has not been firmly related to Cu deficiency in humans, although it clearly does occur in animals (see above). The myocardial changes observed in Cu-deficient animals are quite different from those of ischemic heart disease in humans. This has not deterred Klevay (45) from promoting very strongly his "zinc-copper hypothesis" of ischemic heart disease ever since 1973. He has reviewed his arguments and his papers of the previous ten years (45).

NEUROLOGICAL EFFECTS Neurological effects of mild chronic Cu deficiency should be kept in mind as a possibility, although there is no evidence of such effects to date. If Menkes disease is considered a model, then one might look for Cu deficiency when cerebellar abnormalities are pronounced. The reason for the predominant involvement of Purkinje cells in Menkes disease is not known.

One might anticipate that a deficiency of dopamine-β-hydroxylase might cause disorders of muscle tone and movement. Against this expectation is the report of apparent primary dopamine-β-hydroxylase deficiency in an adult patient with ptosis, hypotonic and orthostatic hypotension, but no neurological or movement disorder (51). The relationship of dopamine-β-hydroxylase to Cu is complex. The enzyme contains Cu, and Cu is also able to block the effect of a tissue inhibitor, at least in vitro. Brains of brindled mice and of Cu-deficient neonatal mice show functional dopamine-β-hydroxylase deficiency, but increased levels of dopamine-β-hydroxylase by assay (42, 75). This background may be relevant when considering the possible effects of Cu deficiency upon motor function and in assessing a recent report of a slight increase in cerebrospinal fluid Cu levels in Parkinson's disease (65).

Methods of Detecting Mild Chronic Copper Deficiency

In looking for methods of diagnosing copper deficiency it is logical to think of measuring the levels of copper in appropriate body tissues and/or measuring the activity of copper enzymes. Of course, the choice of methods must also be influenced by the ease and safety of sampling tissues (86).

It is deficiency in the function of one or more of the Cu enzymes that causes Cu deficiency disease. Thus, it would be ideal to assess the activity of each of the Cu enzymes. Of course, the supply of Cu to an enzyme in one tissue may differ from the supply to the same enzyme in another tissue. Logically, this leads one to argue for assay of all Cu enzymes in the tissue relevant to the

symptoms under study, or in all tissues if a general search for Cu deficiency is being conducted. This is clearly impracticable.

Mere avoidance of deficiency does not guarantee good nutrition. This may require the supply of enough plus a little to spare. For the provision of "a little to spare" to be useful there must be a system of storage and of access to this store when more Cu is needed. Cu-metallothionein may serve this function within individual cells, and Cu-metallothionein in the liver may possibly serve as a store from which Cu is available to other tissues, distributed by ceruloplasmin, but these points are far from established (13).

When choosing tissues in which to measure Cu levels, we are hampered by the inadequacy of our knowledge about the source of the Cu present in each tissue, the degree of interchange of Cu between tissues, the extent of recycling of Cu within a tissue (cell), and the possible existence of a storage form of Cu within individual cells and/or in one tissue as a resource for other tissues.

In practice, we have to choose tissues that can be sampled and that will yield results that can be interpreted. Unfortunately, many studies have employed analyses of tissues that could be sampled (blood, hair, nail) without adequate consideration of the interpretation that must follow.

The liver plays a special role in Cu homeostasis as the organ through which most Cu is excreted from the body and the organ taking up the largest part of Cu after its absorption from the intestine. Liver Cu levels fall more rapidly than the levels of Cu in other organs during Cu deficiency, which suggests that part of the Cu in the liver is available as a store for other organs. Measurement of liver Cu is probably the most sensitive method of demonstrating Cu deficiency, but it is not suitable for most human situations because liver biopsy is too invasive a procedure to use except when there is a compelling clinical reason for assessing Cu status.

When we measure serum (plasma) Cu we are measuring ceruloplasmin because this protein contains over 80% of the Cu present in plasma. Alternatively, we can measure ceruloplasmin itself by immunoassay or by its amine oxidase activity. When we measure ceruloplasmin we are not sure whether we are indirectly estimating a store of Cu in the liver by measuring the molecule that carries Cu from the store to the other tissues (one view of the role of ceruloplasmin) or assessing the adequacy of supply of Cu to a Cu enzyme that happens to be present in the circulation (another view of the role of ceruloplasmin). If, indeed, ceruloplasmin serves both these functions, then its measurement may be combining the two general approaches to diagnosing Cu deficiency. It is probable that ceruloplasmin production is one of the first Cu functions to decline when insufficient Cu is available, but this is not certain. Ceruloplasmin levels do fall in severe Cu deficiency and measurement of either serum ceruloplasmin or serum Cu is adequate to diagnose this condition. A lesser reduction would be expected in mild Cu deficiency, but the

problem is to recognize this reduction against the background variability in normal levels of ceruloplasmin. Ceruloplasmin is an acute-phase responsive protein. Consequently, levels are altered by a variety of intercurrent factors. Its production is stimulated by estrogens (31). Levels are higher in adult females than males, vary during the menstrual cycle, and are greatly elevated during pregnancy (31). Genetic factors (other than the genes concerned in Menkes disease and Wilson disease) also influence ceruloplasmin levels, and benign familial hypoceruloplasminemia has been described (31).

To avoid confusion from these additional sources of variation in ceruloplasmin levels and to identify the influence of Cu deficiency in individual patients, it would be logical to measure ceruloplasmin before and after Cu replenishment, using a physiological replacement dose and an appropriate interval between measurements (15). This general approach is applicable to other enzymes, provided the time interval is adjusted appropriately (15). For this approach to be valid we need to know that ceruloplasmin levels are not raised above normal by excess Cu and to choose the appropriate interval—in this case three days (54).

Red blood cells and white blood cells are also easily available for study. Most of the Cu in red blood cells is present in superoxide dismutase. Since new proteins are not synthesized during the long (200-day) life of red blood cells, and because superoxide dismutase activity cannot be restored by addition of Cu to inactive enzyme (4), one might expect that red cell copper levels would reflect only long-term effects. This would have the advantage of diminishing the confusing influence of intercurrent illnesses, which might be desirable if studying mild chronic deficiency. On the other hand, red cells might be expected to be unsatisfactory for replenishment experiments because one would have to wait for a new population of Cu-replete red cells to be formed. A recent report did support the value of Cu replenishment and measurement of red cell superoxide dismutase in rats, but the interval chosen between measurements was 30 days, too long for clinical use in medicine (97). One might expect an even longer interval to be necessary in humans. Unfortunately, no systematic data are available for humans.

White blood cells contain both superoxide dismutase and cytochrome oxidase and turnover more rapidly. They might be used to assess Cu status if assayed before and after replenishment, but no data are available.

Measurement of urinary Cu excretion cannot help in assessing Cu deficiency because normal levels are already very low. Abnormal urinary losses in certain renal diseases and in TPN can contribute to Cu deficiency.

Measurement of trace element levels in hair and finger nails has become very popular among the proponents of "orthomolecular medicine" and has also been used by scientists. These long-lived tissues are reputed to contain a record of trace element nutrition over preceding months and years. Unfortunately, they also record the assaults of various external agents, including

alkalis in soap, hair dyes, etc, and their measurement is questionable (22, 33, 86).

Returning to assays of copper enzymes in assessing Cu status, it is worth remembering that osteoporosis due to lysyl oxidase deficiency is a prominent feature of severe Cu deficiency in humans. Assay of this enzyme is a cumbersome procedure and requires a skin biopsy. Poorly cross-linked collagen is broken down more rapidly than normal collagen. Some of the breakdown products of collagen are unique (e.g. hydroxylysine) and can be measured in urine. Quantitation of these compounds may provide a useful indicator of Cu deficiency. It should be possible to repeat this measurement after Cu replenishment, if this proved necessary.

DIETARY REQUIREMENTS AND INTERACTIONS

Information available about adult human Cu requirements has been reviewed repeatedly (58, 78, 102). Many balance studies in healthy adults have shown losses of 1.5–2.0 mg per day, mostly in the stools. Therefore intakes of at least 2.0 mg per day have been recommended. A number of nutritional surveys have shown intakes of less than 1.0 mg, which suggests that mild Cu deficiency is very common, or else the requirement stated is overly generous for most people (78).

Although the amount of Cu administered in TPN has varied widely, 0.5 mg per day seems to be an average requirement in adults (102). This fits with the oral requirement if the average efficiency of absorption is about 30%, as has been found in studies with copper isotopes.

Foods with high copper content include animal livers and shellfish, but the principal contributors in an average diet are potatoes, fruit, bread, meats, fish, and legumes (78). Cow's milk and dairy products contain little Cu and that present is poorly absorbable, apparently because it is bound to casein (11). Although the Cu content in human milk is only a little higher than that of cow's milk, the Cu present is well absorbed, most of it being found in the whey (11).

Copper content of breast milk is highest in the early days of lactation and decreases gradually as lactation progresses (9, 26). After premature delivery (26–33 weeks gestation) the Cu content of breast milk is similar to that after term delivery, both in absolute levels and in the progressive changes during lactation (60). Calculations suggested that Cu intake of fully breast-fed premature babies should be sufficient to achieve the rate of Cu accumulation that occurred in utero (60).

The whole situation with regard to Cu and premature babies is quite confusing. It is clear that Cu deficiency is relatively frequent in premature

babies, the symptoms generally developing about 3 or 4 months after birth. It is usually argued that this susceptibility of premature babies to Cu deficiency is related to a "low hepatic Cu store." "Cu stores" in the liver are claimed to accumulate rapidly during the last month or two of fetal life. However, the evidence for an increase in the Cu concentration of the liver during this period is not very convincing (101) and there is no convincing evidence that the Cu present in the liver at birth acts as a "store." That is, the utilization of the Cu from the newborn liver for the formation of Cu enzymes in other tissues has not been demonstrated. This may seem pedantic, but the fact that both Cu incorporation into ceruloplasmin and Cu excretion into the bile are impaired in Wilson disease and that Cu incorporation into ceruloplasmin increases rapidly after birth makes it quite reasonable to suggest that biliary Cu excretion may also increase rapidly after birth. The high copper level in the fetal liver may merely reflect deficient biliary excretion and may not provide a store of Cu for later use.

A number of studies have described the progressive changes in Cu levels over the first two months of life in premature babies; in some studies oral feeding has been used and in others parenteral nutrition. There is clearly a complex interrelationship between the intake of Cu, of Zn, and the growth rate: a high growth rate or high Zn intake increased the requirement for Cu. On the available evidence, it is not possible to describe an ideal combination for the small premature infant, but it is certainly clear that neonatologists should look for Cu deficiency. Some studies have shown a progressive rise in serum Cu (and therefore ceruloplasmin) over the first few weeks of postnatal life in small premature babies (79, 93, 95). Since ceruloplasmin production is not overinduced by excess copper, one must assume that those babies who have failed to show this progressive increase must have been absorbing, or receiving intravenously, less than optimal amounts of Cu, losing it in urine, or sequestering it in an unavailable form (50). When one considers the rather variable pattern of serum Cu in babies receiving TPN, one may need to consider the possibility of Zn/Cu interaction in the liver, as well as in the intestinal mucosa, in addition to urinary losses (94). It is possible that hyperinduction of metallothionein in the liver by Zn could sequester Cu in the liver, rather than making it available for ceruloplasmin synthesis and for other Cu enzymes in other tissues.

Returning to consideration of patients other than premature babies, it is clear that the interaction of Zn with Cu is by far the most important nutritional interaction. It has been discussed sufficiently already.

Protein and dietary fiber content appear to diminish the availability of Cu (78). High dietary intake of ascorbic acid has also been shown to lower serum ceruloplasmin and Cu levels (27). A number of studies have examined the interrelationship between fructose and Cu status. The consensus is that fruc-

tose increases the requirement for Cu, rather than interfering with its absorption. Red cell superoxide dismutase activity was significantly reduced by the high fructose intake, whereas ceruloplasmin levels and serum Cu were not decreased (76).

The influence of intravenous infusion of amino acids has already been mentioned. Other renal diseases, especially those that lead to renal protein loss, may increase Cu requirements, as may protein losing enteropathy. A variety of chronic bowel diseases may also impair Cu absorption.

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