Review

Biochemical changes in copper deficiency

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Introduction

The nutritional trace element copper (Cu) is essential to cellular functioning of all living systems. Biochemical mechanisms have evolved that control the homeostasis of this element to ensure that adequate, but not toxic, levels are absorbed, transported, utilized and excreted. Cu expresses its biological activity while bound to specific protein ligands in microbial, plant, and animal cells. These cuproenzymes are responsible for the biochemical functions of the element Cu.

There are a number of comprehensive reviews dealing with Cu. Owen[1] has written a review of the biochemical aspects of Cu throughout living systems. Details of the molecular properties of several cuproproteins are summarized elsewhere in a collection of works by authors specializing in this aspect of Cu chemistry.[2] Another good resource deals with biological aspects of Cu functions.[3] A recent synopsis listed the well accepted cuproproteins reported in the literature and some that were more controversial.[4] The present contribution will present an overview of the biochemical functions of Cu in animals and will review and emphasize biochemical changes that occur during Cu deficiency.

Biological chemistry

Because of the high avidity of biological ligands for Cu, free ionic Cu^{2+} is present in physiological fluids at extremely low concentrations, in the femtomolar range. Most tissues contain $\sim 5 \times 10^{-5}$ M total Cu. The majority of this Cu is associated with specific proteins, peptides, and amino acids. The biochemistry of Cu in animal cells is defined uniquely by the distribution of Cu containing ligands within them.

In the blood plasma Cu is also largely protein bound principally to ceruloplasmin, an α_2-globulin, and to a lesser degree, to albumin. There is some evidence for an additional plasma cuproprotein called transcuprein.[5] In addition to these proteins, Cu is known to be associated with smaller ligands, peptides, and amino acids. Computer predictions and chemical characterization indicate that, in plasma, Cu is predominantly associated with histidine and to a lesser extent cysteine. Cu also forms ternary complexes with two different amino acids or between Cu^{2+}, histidine, and albumin.[6] Another plasma Cu ligand is the tripeptide glycyl-*L*-histidyl-*L*-lysine (GHL). GHL is a growth modulating peptide that may facilitate Cu uptake into cells.[7]

The cuproproteins of animals are a heterogeneous group of molecules. Neurocuprein is a single peptide chain with one Cu atom.[8] Ceruloplasmin is also a single polypeptide but contain 6–8 Cu atoms.[9] Some cuproproteins, for example copper-zinc superoxide dismutase (Cu,Zn-SOD), are dimers. Dopamine-β-monooxygenase (DBM), is a tetramer. Some contain other metals besides Cu like zinc in Cu,Zn-SOD or heme iron in the polypeptide complex, cytochrome *c* oxidase. Some cuproproteins contain organic prosthetic groups. One such example is plasma amine oxidase which contains pyrroloquinoline quinone (PQQ).[10] The diverse nature of cuproproteins results in a large variety of specialized functions for Cu in biological systems.

Spectroscopic, crystal, and three-dimensional structure work have resulted in the identification and classification of several Cu geometries in a number of proteins. The intense blue color of certain cuproproteins is due the presence of Type I Cu, characterized by a distorted tetrahedron in which EPR detectable Cu^{2+} is associated with two histidines, methionine and cysteine. Type 2 Cu is also EPR detectable and is sensitive to anions. Type 3 Cu is EPR silent due to coupling of a binuclear center of two Cu^{2+} sites. Some cuproproteins such as ceruloplasmin contain all three types of Cu^{2+} sites as well as the less characterized Type 4 Cu^{2+}.[9] Others contain only one type of Cu such as the binuclear Type 3 Cu center in tyrosinase.

Ligand field stabilization energies influence the reduction potentials of the Cu sites in cuproproteins resulting in higher positive potentials than in more simple Cu^{2+} complexes in water.[2] These modified redox properties are essential to the catalytic properties of

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the cuproenzymes. External reducing agents such as ascorbic acid are necessary in some cases, to support catalysis by reaction with Cu^{2+}; in other proteins substrates themselves can reduce Cu^{2+}. Certain cuproenzymes with four metal centers like ceruloplasmin, cytochrome *c* oxidase (2 Cu^{2+} and 2 heme iron), and the plant protein ascorbate oxidase are especially suited to carry out redox reactions with formation of water from molecular oxygen without the intermediate formation of univalent (superoxide) or divalent (hydrogen peroxide) reduction products.

Homeostasis

There is an extensive literature describing Cu metabolism and homeostasis.[11] Uptake of Cu into the small intestine may be facilitated by certain ligands such as amino acids but does not appear to be energy dependent.[12] Absorption can be impaired by certain factors. Dietary zinc, for example, can influence transport from the lumen as well as sequestration within the mucosal cells due to induction of metallothionein (MT), a small cysteine-rich protein.[11,12] It is not established whether intestinal MT is involved physiologically in Cu absorption or transport across the serosal surface.

Cu is transported from the intestine within the portal blood bound to albumin, amino acids, and perhaps to transcuprein.[5,13] Hepatocytes take up Cu from albumin and amino acids such as histidine. The Cu that enters the hepatocyte from portal blood is distributed within several pools. The major efflux pool of this newly entering Cu is ceruloplasmin. Some of the Cu enters the excretory pool destined for bile and some is associated with MT. It is not clear whether liver MT is a storage pool for Cu other than during the neonatal period or during Cu toxicity.[11,14] Some of the incoming Cu from portal blood can be used for functional intrahepatic Cu pools such as Cu,Zn-SOD and cytochrome *c* oxidase.

There is some uncertainty concerning the transport of Cu to extrahepatic organs. A hypothesis that is gaining experimental support suggests that ceruloplasmin (CP) serves this function. Tracer studies indicated that the Cu from CP was found with functional Cu pools and that several organs could take up CP.[15] CP was better able to restore cytochrome *c* oxidase activity in Cu-deficient rats than other Cu donors.[9] Specific CP receptors have been identified.[16] It is not yet clear whether CP is taken up by receptor-mediated endocytosis like transferrin or whether the Cu can get donated to a plasma membrane receptor directly. In nonhepatic tissues evidence for Cu release from CP at the plasma membrane exists.[17] Recent work also supports Cu release from CP at the membrane level.[18] The major unresolved issue is whether Cu uptake from CP is obligatory. For example, CP, Cu-albumin, or $CuCl_2$ were capable of donating Cu to a functional Cu pool (Cu,Zn-SOD) in cultured aortas.[19] Additional experiments are needed to elucidate Cu transport mechanisms.

The excretion of Cu is important to control its homeostasis. This is accomplished largely by biliary secretions since much less Cu is excreted in the urine. Little enterohepatic recirculation occurs beyond the neonatal period. Circulating CP can be removed by liver first, by desialation in the endothelium, and then via the galactosyl-recognition system in hepatocytes.[20] If biliary Cu excretion is not functioning optimally, hepatic toxicity of Cu can be observed as is in Wilson's disease (a genetic disorder) and biliary cirrhosis.[21] The liver is the key organ regulating Cu homeostasis.

Cuproenzymes

Extrahepatic organs have a large number of diverse cuproproteins that are responsible for the bulk of the biochemical functions of Cu. The well established biochemical functions of Cu are due to specific cuproenzymes. Some of these enzymes such as Cu,Zn-SOD and cytochrome *c* oxidase are ubiquitous enzymes underscoring the importance of Cu in all animal cells. Other cuproenzymes have more specialized functions and are unique to certain cells. Dopamine-β-monooxygenase is found only in noradrenergic neurons; and tyrosinase only in melanocytes. Nevertheless, the biochemical functions of Cu, as currently known, are due to specific cuproenzymes (*Table 1*). Many proteins have been isolated and reported to contain Cu following the establishment of Cu essentially in the 1920's. Several of these proteins have been well characterized and the Cu stoichiometry established and documented by many research groups.[1] Included among these enzymes are ceruloplasmin, cytochrome *c* oxidase, dopamine-β-monooxygenase, Cu,Zn-SOD, and tyrosinase (*Table 1*).

Much confusion exists concerning the role of Cu in amine oxidases.[22] It is now well established that certain amine oxidases do contain Cu, namely that group (EC 1.4.3.6) once classified as "diamine oxidases" based on their catalytic preferences. This group of enzymes known also as plasma amine oxidase, benzyl amine oxidase, kidney diamine oxidase, and lysyl ox-

Table 1 Established cuproenzymes of animals

Enzyme	Functions
Amine oxidase	Oxidative deamination
Ceruloplasmin	Copper transport, Fe^{2+} oxidation
Cytochrome c oxidase	Electron transfer
Diamine oxidase	Oxidative deamination
Dopamine-β-monooxygenase	Norepinephrine synthesis
Extracellular superoxide dismutase	Dismutation of O_2^-
Lysyl oxidase	Elastin and collagen synthesis
Metallothionein	Copper storage
Neurocuprein	Modulate monooxygenase activity
Peptidylglycine α-amidating monooxygenase	C-terminal peptide modification
Cu,Zn-superoxide dismutase	Dismutation of O_2^-
Tyrosinase	Melanin synthesis

idase are very similar, yet not identical, in molecular properties (*Table 1*). All these animal proteins appear to be dimers of molecular weight ~ 150,000 to 200,000 each containing 2 moles of type 2 Cu, likely 1 per each subunit. In addition, these proteins probably all contain the organic prosthetic group pyrroloquinoline quinone (PQQ). This coenzyme was once thought to be pyridoxal phosphate. Other amine oxidases may exist such as the Cu-manganoprotein isolated from human placenta.[23] However, earlier reports that mitochondrial monoamine oxidases (EC 1.4.3.4) were also Cu containing enzymes are probably not true.[4] These enzymes which are not restricted to primary amines as substrates are flavoproteins.

Several less well characterized cuproenzymes exist (*Table 1*). Marklund[24] has described an extracellular form of superoxide dismutase (EC-SOD). This enzyme has four subunits, four Cu and zinc atoms. The estimated molecular weight is similar to that of ceruloplasmin. It is not inhibited by antibodies against Cu,Zn-SOD, or CP. EC-SOD is made by selected anchorage-dependent cells and may be important in removing superoxide from the extracellular space.[25] A relatively new cuproenzyme that has not yet been purified sufficiently enough to establish the Cu stoichiometry is peptidylglycine α-amidating monooxygenase (PAM). The activity, like dopamine-β-monooxygenase, requires oxygen, and ascorbate.[26] This new cuproenzyme is involved in the synthesis of many bioactive peptides that are posttranslationally modified. It represents an additional neurochemical function dependent on Cu.[27]

Another cuproprotein called neurocuprein has been characterized in cells involved with norepinephrine synthesis.[8] This acidic protein, found in brain and adrenal glands, may have a role in modifying catecholamine metabolism. Its true enzymatic function remains to be established. The last well established cuproprotein is metallothionein (MT). Metallothionein, like neurocuprein, may not really be classified as a true cuproenzyme. There are numerous reviews on structure and expression of MT.[1,11,12] Metallothionein is a small single polypeptide containing 61 or 62 amino acids, 20 of which are cysteine. It folds into two distinct domains. The α-domain binds preferentially zinc whereas Cu is found in the β-domain. A physiological MT containing both zinc and Cu could potentially have distinct functions because of this unique binding preference. However, despite our knowledge about the biological chemistry of MT and its genetic expression, little is firmly established concerning MT functions, especially regarding Cu. Potential roles include an obligatory pool for hepatic Cu homeostasis, detoxification of Cu, antioxidant activity, or donation to apocuproenzymes during synthesis. Further work will be needed to establish the physiological function of Cu-MT.

Putative cuproproteins

Many other cuproproteins have been described in the literature; some are not enzymes and others, that are enzymes, have no apparent need for the Cu attached (*Table 2*). Plasma contains specific proteins that are involved in Cu transport from intestine to liver, albumin, and transcuprein.[5] Another cuproprotein called ferroxidase II is a large plasma lipoprotein which catalyzes the oxidation of Fe^{2+} to Fe^{3+} but is not inhibited by azide, a characteristic of ceruloplasmin (ferroxidase I). The size and Cu content have been difficult to establish.[28] This may be because most of the Cu is nonspecifically bound.[29] Plasma also contains Cu proteins with no known functions including coagulation factor V and an IgG_1 from multiple myeloma patients.[30,31]

Table 2 Putative cuproproteins of animals

Protein	Reported location
Albocuprein I	Brain
Albocuprein II	Brain
Albumin	Plasma
Coagulation factor V	Plasma
Ferroxidase II	Plasma
Guanylate cyclase	Lung
Lipolytic protein	Liver
Metabolite binding protein	Bile
Myeloma protein (IgG_1)	Plasma
Pink protein	Erythrocytes
Transcuprein	Plasma

There are also a number of intracellular cuproproteins (*Table 2*). Some of these are restricted to one particular organ or cell type. In the brain, for example, two cuproproteins were described, albocuprein I and II, whose enzymatic functions, if any, are not known.[32] Other examples of cuproproteins with no known functions are a pink protein isolated from human erythrocytes[33] and a rat biliary protein that also binds azo dye metabolites.[34] Two additional proteins have been described that contain Cu; these are the enzymes guanylate cyclase from bovine lung[35] and a zinc-Cu lipolytic protein from chick liver.[36] It is not known if Cu plays a role in catalysis in these two putative cuproenzymes.

Manuscripts have been published which described enzymes that were isolated and found to contain one or more metals. Upon further purification and implementation of additional procedures aimed at removing metals, the enzyme in question would retain full activity without the presence of metal. This scenario has certainly been true for Cu. Because Cu has a strong affinity for protein it is not surprising that many isolation protocols result in the presence of adventitious Cu in the final product. However, when the concentration of Cu is below a reasonable stoichiometric value the definition of "cuproenzyme" should be reviewed with skepticism. There are many examples of questionable cuproproteins.[1] In some cases the data are quite convincing both pro and con.

Several examples of controversial cuproenzymes have recently been documented.[4] This list includes: (1) δ-aminolevulinate dehydratase; (2) Cu-chelatin; (3) cysteamine dioxygenase; (4) β-mercaptopyruvate

transsulfurase; (5) mitochondrial monoamine oxidase; (6) tryptophan 2,3-dioxygenase; and (7) uricase. Perhaps other proteins that are currently thought to contain Cu (*Table 2*) will be added to this list. The establishment of true cuproenzymes will be facilitated by improving analytical techniques for measuring Cu and the availability of high purity reagents for use in protein isolation.

Cu deficiency: pathological and biochemical changes

Cu deficiency in experimental animals (principally rodents), domestic animals, and humans has been studied for many years. During that time, changes to many organs have been noted.[3] The appearance of these changes is both a function of the severity of the Cu deficiency and the species involved. When severe, Cu deficiency impairs growth and reproduction. Some of the most salient changes to organs include alterations in the hematological, cardiovascular, nervous, and immune systems (*Table 3*). For example, anemia, cardiac enlargement, neuronal degeneration, and thymic hypoplasia have been noted in a number of species. Other changes are more species specific. For example, splenomegaly was observed in Cu-deficient mice but not rats.[37] Some organs have received little attention regarding pathological changes following Cu deficiency including adrenal gland, skeletal muscle, and the gonads. In some organs only selected cell types are altered. A most remarkable pictorial documentation of this was shown in the loss of pancreatic acinar cells with retention of a relatively normal endocrine pancreas.[38]

There have been many biochemical measurements performed on tissues and fluids obtained following Cu deficiency. Some measurements have been verified by more than one research team and are generally accepted (*Table 4*). Other measurements have been made infrequently or have not been consistent among researchers. Nevertheless, a number of biochemical alterations have been reported. Some of the changes have been reported in a number of species such as the alterations in Cu levels, lower hemoglobin, reduced melanin, and elevated hepatic Fe content.[3] For the most part, the molecular etiology of these biochemical changes has not been established.

Table 3 Organ pathology following copper deficiency

Organ	Pathological observation
Adrenal	Mild change
Blood	Anemia, neutropenia
Bone	Decreased osteogenesis, osteoporosis
Brain	Neuronal degeneration, mitochondrial hypertrophy
Gonads	Mild change
Heart	Marked enlargement, mitochondrial hyperplasia
Intestine	Diarrhea
Kidney	Mild atrophy
Liver	Mild hepatomegaly
Lung	Emphysema
Skeletal muscle	Mild change
Pancreas	Atrophy, loss of acinar cells
Skin	Achromotrichia, altered keratinization
Spleen	Splenomegaly, altered lymphocyte distribution
Thymus	Hypogenesis, necrosis

Table 4 Biochemical observations following copper deficiency

Generally accepted	Somewhat controversial
Lower organ Cu levels	Impaired energy metabolism
Higher liver Fe levels	Lower plasma ascorbate
Lower hemoglobin levels	Hyperuricemia
Hypercholesterolemia	Lower brain dopamine
Hypertriglyceridemia	Hypoferremia
Glucose intolerance	Elevated hepatic glutathione
Elevated organ dopamine	Elevated organ lactate
Lower organ norepinephrine	Altered fatty acid composition
Decreased melanin	Endogenous lipid peroxidation

Table 5 Altered cuproenzymes following copper deficiency

Enzyme	Evidence for rate limiting pathway
Amine oxidase	None
Ceruloplasmin	Elevated hepatic Fe (controversial)
Cytochrome c oxidase	Weak
Dopamine-β-monooxygenase	Higher dopamine, lower norepinephrine
Extracellular superoxide dismutase	None
Lysyl oxidase	Impaired crosslinking of elastin
Peptidylglycine α-amidating monooxygenase	None
Cu,Zn-superoxide dismutase	None
Tyrosinase	Hypopigmentation

It is not surprising, since most of the Cu in the cell is associated with enzymes, that much attention has been directed at measuring cuproenzymes following Cu deficiency. Even though the activity of many cuproenzymes measured in vitro is lower following Cu deficiency, rarely does it directly follow that the change is sufficient to alter the flux of a metabolic pathway (*Table 5*). Plasma amine oxidase activity was shown to be altered by Cu deficiency in 1965 but the significance of that observation remains unknown.[39]

Owen[1] has written extensively concerning CP, the changes during Cu deficiency, and the theory regarding ferroxidase activity. There are still uncertainties about the relationship of CP to normal Fe metabolism and heme synthesis. In mice, for example, the ferroxidase theory does not seem to hold.[40] Further studies indicate that anemia precedes elevated hepatic Fe by 3 weeks in neonatal Cu-deficient mice.[41]

Two enzymes involved in dismutation of superoxide both require Cu for activity and have been measured following Cu deficiency. Cytosolic Cu,Zn-SOD activity was first shown to be lower in brains of Cu-deficient rats by Prohaska and Wells[42] although no evidence of abnormal lipid peroxidation was detected.[43]

DiSilvestro[44] has reported lower extracellular SOD activity following Cu deficiency in rats.

Peptidylglycine α-amidating monooxygenase (PAM) was evaluated indirectly in Cu-deficient rats by measuring levels of anterior pituitary α-MSH.[45] The precursor to this peptide is one modified by PAM. No changes were observed; however, that single study began with rats which weighed 100 g and the brain Cu at the end of the study in the "Cu-deficient" group was 1.8 μg/g, not markedly lower than the control value of 2.5.

One of the first cuproenzymes shown to be altered by Cu deficiency was cytochrome *c* oxidase.[46] However, attempts to demonstrate that lower activity is associated with altered energy metabolism have been less than impressive. Brain ATP was not lower in Cu-deficient weanling rats that had a 70% reduction in cytochrome *c* oxidase activity.[43] In brains and hearts of suckling mice with greater than 80% reduction in cytochrome *c* oxidase activity adenine nucleotide energy charge was not altered.[47] In hearts of young adult Cu-deficient mice and rats, modest changes in adenine nucleotides have been reported.[47,48]

Of the many cuproenzymes whose activity changes have been documented following Cu deficiency three (dopamine-β-monooxygenase (DBM), lysyl oxidase, and tyrosinase) have been shown by several groups to be rate limiting following Cu deficiency. DBM activity was first shown to be altered by Cu deficiency in hearts of rats by measuring conversion of radioactive dopamine (DA) to norepinephrine (NE). The steady-state level of NE was first shown to be lower following Cu deficiency by Prohaska and Wells[42] and then subsequently by others especially by O'Dell and co-workers.[49] Recently, a study was completed that showed clearly that DBM was rate limiting in that elevated DA and lower NE were quantified in several organs from both Cu-deficient mice and rats.[37] It is more difficult to demonstrate impaired DBM enzyme activity in vitro from Cu-deficient animals.[50]

Impaired lysyl oxidase activity explains a major part of the pathophysiology of Cu deficiency. In fact the altered elastin and collagen molecules may secondarily alter many organ systems of Cu-deficient animals. O'Dell and co-workers investigating Cu-deficient chickens first demonstrated the connection between impaired enzyme activity and altered connective tissue.[51]

Evidence that tyrosinase was rate limiting in Cu deficiency was first made by observing the altered pigmentation in laboratory rats and sheep grazing on pastures low in Cu.[3] In the laboratory tyrosinase activity was also shown to be altered by dietary and genetic copper deficiency.[52] Pigmentation is altered in humans with Menkes' disease, an inherited X-linked disorder characterized by Cu deficiency in certain cells.[21] In summary, some of the known pathological changes observed in Cu deficiency (*Table 4*) can be explained by alterations in known cuproenzymes (*Table 5*); however, most of the biochemical changes have an unknown and multiple etiology.

Noncuproenzymes altered by Cu deficiency

When Cu becomes limiting in a cell, a number of enzymes known not to contain stoichiometric levels of Cu, have been described that have altered activity in vitro (*Table 6*). In some cases these changes in enzyme activty have not been confirmed upon further experimentation. In other cases the changes can be explained by logical interpretation. For some enzymes it is not clear which mechanism has resulted in altered activity. It is also not clear whether these noncuproenzymes are influenced by modest fluctuations in cellular Cu homeostatis or whether the changes in enzyme activity become rate controlling in metabolic flux. Perhaps some of the biochemical functions of Cu are mediated indirectly by these noncuproenzymes (*Table 6*).

A number of enzymes have been reported to be higher in enzyme activity when studied from Cu-deficient animals. For example, the activity of plasma membrane alkaline phosphodiesterase I from spleen and thymus was shown to be higher in lymphocytes from Cu-deficient mice.[53] In Cu-deficient rats activity of microsomal heme oxygenase, mitochondrial succinate dehydrogenase (SDH), plasma GOT, glucose-6-phosphate dehydrogenase, and hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co A) reductase were shown to be higher compared to control rats (*Table 6*). Some of these changes can be explained. The elevation in heart SDH activity is likely due to the increased mitochondrial numbers in the Cu-deficient myocardium.[54] There have been others, however, who have found lower heart SDH activity in Cu-deficient rats.[55] SDH activity in other organs of Cu-deficient rats, like liver[56] and brain[43] are not different from controls. Plasma GOT elevation likely reflects liver damage in severe cases of Cu deficiency.[57] The elevation in hepatic HMG Co A reductase in Cu-deficient rats ob-

Table 6 Noncuproenzymes whose activities change following copper deficiency

Enzyme activity	Change	Reference
Alkaline phosphodiesterase I	Higher	53
Aniline hydroxylase	Lower	65
Catalase	Lower	76
2,3'Cyclic nucleotide-3'-phosphodiesterase	Lower	42
Glucose 6-phosphatase	Lower	64
Glucose 6-phosphate dehydrogenase	Higher	82
Glutathione peroxidase	Lower	77
Glutathione transferase	Lower	79
HMG-CoA reductase	Lower	58
Glycerolphosphate acyltransferase	Lower	68
Heme oxygenase	Higher	66
Latent hexokinase	Lower	61
Lecithin:cholesterol acyltransferase	Lower	71
Lipoprotein lipase	Lower	70
Glutamic oxaloacetic transaminase	Higher	57
Stearyl-CoA desaturase	Lower	73
Succinic dehydrogenase	Higher	54
Tyrosine hydroxylase	Lower	62
UDP-Galactose:galactosyl transferase	Lower	60

served by Valsala and Kurup[58] has recently been confirmed by others.[59] Perhaps the elevated activity is the result of lower hepatic cholesterol levels in Cu-deficient rats, since it is known that cellular cholesterol levels affect HMG-CoA reductase synthesis.

Most examples of altered enzyme activity following Cu deficiency involve lower activity (*Table 6*). In certain organs these enzyme changes are related to delayed maturation. The brain of Cu-deficient rodents is a good example.[49] Offspring of Cu-deficient rat dams exhibit hypomyelination. Thus activity of two proteins known to be associated with myelin, 2′,3′-cyclic nucleotide 3′-phosphodiesterase[42] and UDP-galactose: hydroxyfatty acid-ceramide galactosyl transferase,[60] has been shown to lower in brains of Cu-deficient rats. The activity of latent hexokinase in developing cerebella of Cu-deficient rats is also lower than controls, again reflecting delayed maturation rather than Cu requirement.[61] Brain of Cu-deficient rats also showed lower activity of tyrosine hydroxylase,[62] perhaps reflecting neuronal loss since it is known this enzyme requires iron not Cu. The brains of less Cu-deficient rodents (brindled mice) did not have lower activity of tyrosine hydroxylase; in fact, it was higher.[63]

Cu deficiency in rats is accompanied by changes in several liver microsomal enzymes but in an inconsistent manner. One group[64] found lower activity of glucose-6-phosphatase in Cu deficient rat liver while others[65] did not. Content of hepatic microsomal cytochrome P_{450} has been shown to be unchanged in Cu-deficient rats.[66] However, it has been reported that liver aniline hydroxylase activity is lower in Cu-deficient rats compared to normal rats.[65] However, the same study reported an increase in the ability to hydroxylate benzpyrene (another type I substrate). Recently, another lab has shown a reduction in P_{450} activity by following demethylation of aminopyrine.[67] Clearly, the effects of chronic Cu deprivation on microsomal enzymes needs further research.

A number of enzymes involved in lipid metabolism have been studied in Cu-deficient rodents and some of these change following dietary Cu deficiency (*Table 6*). It was once believed that alteration in phospholipid metabolism in Cu deficiency was due to lower activity of glycerolphosphate acyltransferase[68] but this is probably not true.[69] Two groups have reported that lecithin:cholesterol acyltransferase (LCAT) activity is lower in plasma of Cu-deficient rats,[70,71] however, this has been questioned by others.[72] Lipoprotein lipase activity has been reported to be lower in Cu-deficient rats.[58,73] Rat microsomal activity of stearyl-CoA desaturase was shown to be lower in liver from Cu-deficient rats.[74] This same study showed the expected decrease in the ratio of oleic to stearic acid in Cu-deficient rats. Others, however, have not consistently seen changes that would reflect lower delta-9 desaturase activity.[53,75]

The role of Cu in Cu,Zn-SOD has prompted studies into the effects of Cu deficiency on lipid peroxidation.[43] In the course of these experiments other "antioxidant" enzymes have been measured. The activity of the selenoenzyme glutathione peroxidase (GSH Px) has been reported to be lower in liver of Cu-deficient rats.[76,77] In Cu-deficient mice liver GSH Px changes little.[78,79] In contrast, Cu-deficient mice have lower hepatic GSH transferase activity whereas Cu-deficient rats do not (unpublished data).[79] Catalase activity has been reported to be lower in liver of Cu-deficient rats; however, the rats in that study may also have been iron deficient.[76] Others, for example, have found no change in catalase activity following dietary Cu deficiency.[56,79]

Due to the extreme atrophy of the exocrine pancreas, a number of pancreatic enzymes display lower activity following Cu deficiency.[80] However, following dietary Cu deficiency the majority of cellular enzymes remain unchanged in activity[56] even in liver[81] and brain.[49] Those that do change consistently are cuproenzymes. Those noncuproenzymes which change have no consistent pattern; however, it is interesting to note that a number of them are Fe-dependent (cytochrome P_{450} activity, catalase, heme oxygenase, stearyl-CoA desaturase, succinic dehydrogenase, and tyrosine hydroxylase). Further research will be required to determine if this is coincidental or rather is an expression of a Cu-Fe interaction. The fact remains that most of the biochemical functions of Cu can be explained by known cuproenzymes.

Organ specificity

It makes sense physiologically that when Cu is limiting in the diet that some preference be given for Cu distribution to organs and subcellular organelles. This preference is known to be dependent on factors such as age, species, and sex in addition to the organ. In the postweanling rat model it is known that certain organs are more sensitive than others to Cu deficiency.[37,83] The brain is the organ least depleted of Cu in this model whereas heart, liver, and spleen, in one recent study, contained only one-fifth the level of Cu measured in Cu-adequate rats.[37] In a rat model in which Cu deficiency was employed during gestation and lactation brain Cu was markedly depleted.[42]

Other species also exhibit organ specificity following dietary Cu deficiency. An experimental paradigm has been employed involving both inbred and outbred mice studying Cu deficiency during lactation and postlactation. The organs most depleted of Cu of the eight examined in Swiss albino mice studied at 6 weeks included the cerebellum, spleen, and heart while liver Cu was somewhat spared.[37] In other studies with suckling mice the liver Cu depletion was more marked with a mean value in the Cu-deficient offspring < 5% of the Cu-adequate group.[84] This demonstrates that the timing of Cu deficiency also influences the resultant effects on organ Cu distribution.

Cuproenzyme specificity

When Cu becomes limiting which cuproenzyme is altered first? This is an interesting and important ques-

tion that has not yet been fully answered. Some studies have been done comparing two important intracellular cuproenzymes Cu,Zn-SOD and cytochrome *c* oxidase. Paynter and co-workers have measured these two enzymes in seven organs and red blood cells of postweanling Cu-deficient rats.[83] After 6 weeks on the diet Cu,Zn-SOD activity was lower in red blood cells and in five organs. Brain and muscle Cu,Zn-SOD activity showed no differences in this model. Cytochromic *c* oxidase activity was not different in brain or lung but was lower in the other five organs. The relative drop in each enzyme was calculated (percent Cu-supplemented) and the ratio of the change in Cu,Zn-SOD activity compared to cytochrome *c* oxidase activity was determined (*Figure 1*). It is apparent that organ specificity exists for rats. There is a much larger drop in Cu,Zn-SOD activity in liver than in other organs. In contrast, for heart and muscle, cytochrome *c* oxidase activity is more altered relative to Cu,Zn-SOD. Changes in the two enzymes in the other organs were relatively similar as evidenced by a ratio close to 1.0 (*Figure 1*). Paynter and co-workers also showed that the differential loss of activity occured consistently during the development of Cu deficiency in rats.[83] In liver, for example, the drop in Cu,Zn-SOD was greater than cytochrome *c* oxidase as early as 1 week after the onset of Cu deficiency; the reverse enzyme pattern was observed for heart and muscle.

The effects of species differences regarding response to Cu deficiency are evident when contrasting the results of rat studies to those of mice. Postweanling Cu deficiency in albino mice using a dietary protocol similar to that of Paynter *et al.* resulted in the development of Cu-deficient mice with many features similar to the rat.[85] However, one notable difference was the response of liver to Cu depletion regarding Cu,Zn-SOD and cytochrome *c* oxidase (*Figure 2*). In

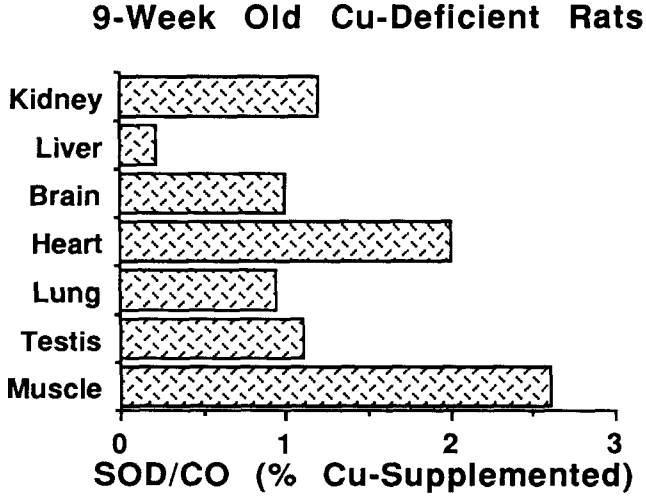

Figure 1 Ratio of residual Cu,Zn-superoxide dismutase (SOD) activity to cytochrome c oxidase (CO) activity in organs from Cu-deficient rats. Data were calculated from activities reported by Paynter *et al.*[83]

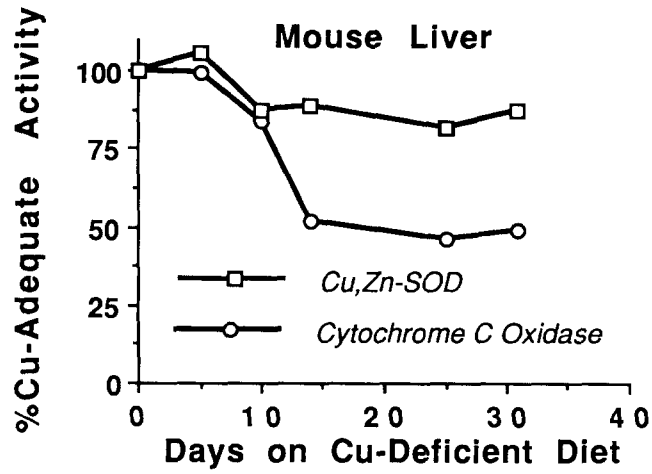

Figure 2 Residual enzyme activity following onset of dietary Cu deficiency in weanling male albino mice. Each point is the average loss measured in three mice. Adapted from mice described earlier.[85]

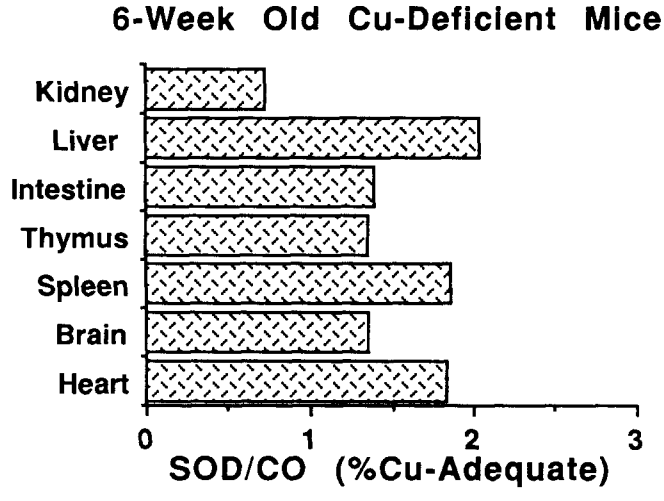

Figure 3 Ratio of residual Cu,Zn-superoxide dismutase (SOD) activity to cytochrome c oxidase activity (CO) in organs from Cu-deficient male Swiss albino mice. Production of Cu deficiency and other features of this treatment have been described elsewhere.[37]

mice cytochrome *c* oxidase activity is more sensitive to Cu deprivation than is Cu,Zn-SOD. A more detailed comparison of other mouse organs, in a manner similar to that presented for rat, shows that except for the kidney the loss of cytochrome *c* oxidase activity is greater than that of Cu,Zn-SOD (*Figure 3*). For heart, liver, and spleen (for which the ratio approached 2.0) this was more noticeable than for the brain, intestine, and thymus.

When similar results were calculated for suckling mice a similar pattern emerged (*Figure 4*). In this case the major organs that show the disparity between loss of Cu,Zn-SOD and cytochrome *c* oxidase were the heart and brain.[84] However, with the single exception of bone marrow, the loss of cytochrome *c* oxidase activity was greater than the loss of Cu,Zn-SOD activity.

The mechanisms that control the intracellular distribution of Cu are not known. The reason that cyto-

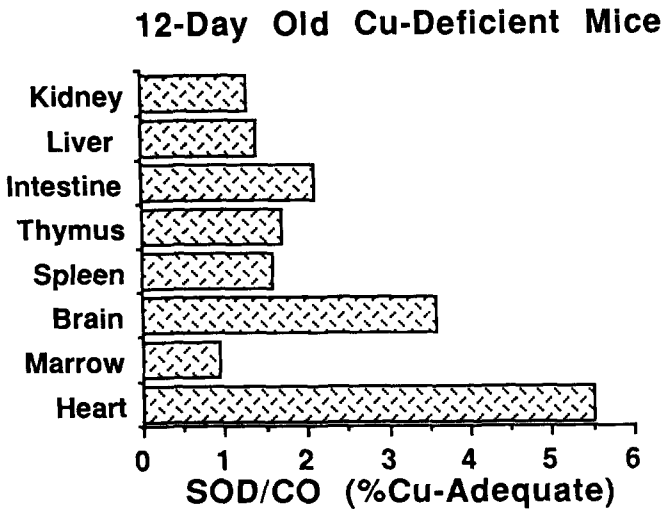

Figure 4 Ratio of residual Cu,Zn-superoxide dismutase (SOD) activity to cytochrome c oxidase (CO) activity in organs from Cu-deficient mice. Data were calculated from activities reported by Prohaska.[84] Other features of these mice including organ weights and Cu levels were described.

chrome *c* oxidase activity responded differently in the liver compared to the heart, for example, may be related to the known differences in the subunits of this polypeptide complex in different organs. There is greater interspecies homology within an organ than between organs in a single species.[86] The response of Cu,Zn-SOD to Cu deficiency is unclear. Others have noted that only modest changes occur in rat Cu,Zn-SOD activity even when 90% of the hepatic Cu was gone.[87] However, in my work liver Cu,Zn-SOD activity from Cu-deficient rats fell to 11% of the level measured in Cu-adequate rats whereas the heart at 46% and kidney at 75% were less affected (unpublished data). These changes are similar to those observed by Paynter *et al.*[83] Cu,Zn-SOD seems to be more vulnerable than cytochrome *c* oxidase to Cu deprivation in rat liver. It is interesting to note that Cu deficiency has no apparent effect on the production of Cu,Zn-SOD protein as the apoprotein was detected in the rat liver of Cu-deficient rats.[87]

There is much to be learned concerning the metabolic handling of Cu by liver and other organs and the response to limitations in Cu flux. A useful approach has been the experimental rodent. Comparative nutritional studies and the use of mutants with altered Cu homeostasis such as mottled mice should facilitate a better understanding of the biochemical changes that accompany Cu deficiency. A note of caution should be made for those using this approach. In addition to the variables mentioned above such as organ, age, and species differences, major variations within a species are known to exist. Suttle and Jones have recently described an excellent example of variable resistance to infection within species of sheep.[88] Mice also express large strain differences. Many studies of deficiency have been conducted with a variety of mouse strains using a similar protocol and diet (modified AIN-76A). Combined gestational and lactational Cu deficiency in C3H/HeJ mice results in offspring that are not altered in growth.[40] In contrast, in C58 mice the offspring die midway through lactation.[89] The immune system of C58 mice is more sensitive to Cu deprivation compared to C57BL or Swiss albino mice.[90,91] Suckling C57BL Cu-deficient pups are much smaller than their Cu-adequate counterparts whereas there is no weight difference between groups in C3H/HeJ offspring.[50] Hopefully, researchers will take advantage of these observations and employ these genetic variations to enhance our understanding of Cu metabolism and the biochemical changes that occur when Cu is limiting.

Relevant questions

During the past seven decades much as been learned concerning the biochemical effects of Cu deficiency. However, despite the confirmed documentation of many observations, in only a few cases can the molecular etiology be traced to a known cuproenzyme. Examples that illustrate a relationship are: the altered connective tissue because of lower lysyl oxidase and hypopigmentation because of reduced tyrosinase. However, well documented phenomena such as altered red and white blood cell distribution, cardiac enlargement, pancreatic acinar cell loss, and neuronal degeneration can not be explained by reduction in activity of a specific cuproenzyme. Similarly, hypohemoglobinemia, hypercholesterolemia, elevated brain lactate, and lower organ norepinephrine can not be explained definitively by reduction in unique cuproenzymes. Thus, the etiology of most of the pathophysiological changes associated with Cu deficiency remains to be established.

More fundamental issues arise that concern the molecular events when Cu is taken into cells and incorporated into storage and functional pools. Is there a unique intracellular Cu binding protein which serves to direct incoming Cu to the proper site? Does intracellular Cu influence transcription of genes coding for cuproenzymes? How does Cu transfer to newly translated apoproteins? The answer to these and other related questions should serve to stimulate biochemical and nutritional research on this essential trace metal.

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