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REVIEW

Hohenheim Consensus Workshop: Copper

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Copper (Cu) is an essential trace element with many physiological functions. Homeostatic mechanisms exist to allow Cu to act as a cofactor in enzymatic processes and to prevent accumulation of Cu to toxic levels. The aim of this commentary is to better understand the role of dietary Cu supply in deficiency and under physiological and pathological conditions. The essentiality of Cu can be attributed to its role as a cofactor in a number of enzymes that are involved in the defence against oxidative stress. Cu, however, has a second face, that of a toxic compound as it is observed with accumulating evidence in hepatic, neurodegenerative and cardiovascular diseases. The destructive potential of Cu can be attributed to inherent physico-chemical properties. The main property is its ability to take part in Fenton-like reactions in which the highly reactive and extremely deleterious hydroxyl radical is formed. Diseases caused by dietary Cu overload could be based on a genetic predisposition. Thus, an assessment of risk-groups, such as infants with impaired mechanisms of Cu homeostasis regarding detoxification, is of special interest, as their Cu intake with resuspended formula milk may be very high. This implies the need for reliable diagnostic markers to determine the Cu status. These topics were introduced at the workshop by the participants followed by extensive group discussion. The consensus statements were agreed on by all members. One of the conclusions is that a re-assessment of published data is necessary and future research is required.
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1. What is the physiological function of Cu?

> 'There are a number of functional Cu-containing enzymes and storage proteins. Some of these have highly specific and some have non-specific functions.'

For more than 80 y Cu has been recognised as an essential nutrient in man. The rapid development in the analytical field and in cell biology provided an increasing understanding of the role of Cu in immune defences, connective tissue and skeleton, blood formation, the blood vessels and nervous system. Behind iron and zinc, Cu is the third most common trace element in the human body. The adult organism contains about 4 g of iron and 80–100 mg Cu (Halliwell & Gutteridge, 1985; Harris, 1991). The average Cu concentration in the cerebrospinal fluid is 70 μM and 250 μM in the synaptic cleft (Kardos *et al*, 1989; Linder & Hazegh-Azam, 1996).

Cu is an integral part of a number of enzymes. For their function they require either Cu alone or Cu and other metal ions as co-factors. Such enzymes are Cu/Zn-superoxide dismutase (Cu/Zn-SOD), ceruloplasmin, cytochrome oxidase, tyrosinase and lysine oxidase. In addition, Cu-dependent transcription factors also play an important part in gene expression. The enzymes in which Cu is an intrinsic component of the catalytic centre are members of the oxidoreductase family. These enzymes are essential for cellular respiration, defence against free radicals, melanin synthesis, formation of connective tissue and iron metabolism (Table 1). The role of Cu in Cu/Zn-SOD has been intensively studied. SOD catalyses the dismutation of superoxide

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Table 1 Some Cu-dependent enzymes and their functions

Cu/Zn-superoxide dismutase	Antioxidative defence
Ceruloplasmin/ferroxidase	Fe binding to transferrin
Cytochrome oxidase	Cellular respiration
Tyrosinase	Melanin synthesis
Lysin oxidase	Formation of connective tissue

anions in the cytoplasm, which is a by-product of cellular respiration. During dismutation, Cu in the active centre of SOD is reduced by the substrate O_2^-; the resulting metabolites are O_2 and H_2O_2. In this reaction Cu cannot be replaced by any other metal. Genetic mutations of SOD1 that alter the enzyme go along with increased apoptosis of neuronal cells, eg in amyotrophic lateral sclerosis (ALS).

Iron and Cu metabolism are interrelated via the ferroxidase activity of ceruloplasmin. In subjects with inherited ceruloplasmin-deficiency the lack of ferroxidase activity decreases the conversion of Fe^{2+} to Fe^{3+} and, consequently, iron binding to transferrin (Gittlin, 1998). The consequence is a disturbed iron distribution. Either iron deficiency (Wang *et al*, 1995) or iron excess were observed, leading to neurodegenerative sequels in certain areas of the brain (Gittlin, 1998). In addition, ceruloplasmin has antioxidative functions as it reduces Fe^{2+}-dependent oxidative stress (Gutteridge, 1980). In contrast, Cu ions bound to albumin and free amino acids are able to participate in Fenton reactions themselves and convert reactive oxygen species like H_2O_2 and O_2^- to $^{\bullet}OH$ radicals (Brigelius *et al*, 1974). Cu binding to viruses, DNA, carbohydrates, enzymes and other proteins, such as amyloid, can cause local damage of these compounds as the result of local $^{\bullet}OH$ formation (Multhaup *et al*, 1998; Quinlan & Gutteridge, 1988). These observations suggest that reactive oxygen species *in vivo* may only be toxic in the presence of catalysing metal ions. In addition to its role in the catalytic centre of Cu-dependent enzymes, there is increasing evidence that Cu is also engaged in gene expression of various proteins. Cu bound to transcription factors is involved in the modulation of eukaryotic gene expression by activation or repression of genetic transcription.

2. How much Cu is absorbed from the diet; where and how is Cu absorbed in the gastrointestinal tract?

> 'Dietary copper is absorbed in the stomach (small amounts), duodenum and proximal jejunum. About 20–70% of dietary Cu is absorbed. In adults maximum absorption is 50%; in infants it is 77%. Absorption can be strongly affected by ligands and other metals.'

The kind of ligands as well as the size, solubility and stability of the Cu complexes formed in the intestinal lumen, have a strong influence on Cu bioavailability. In contrast to other metals, Cu is absorbed in the stomach. This is of minor importance, though, because food is incompletely digested at this location. Cu absorption involves active saturable transport mechanisms as well as diffusion (Crampton *et al*, 1965). The absorptive capacity for Cu is comparable in all sections of the small bowel. In contrast to adult animals, the immature Cu absorption process in rat pups is not saturable (Varada *et al*, 1993) and has a greater transportation capacity (Lönnerdal *et al*, 1985). It is predominantly mediated by diffusion and by co-transport with water (solvent drag). Correspondingly, a lack of sodium in the lumen or inhibition of intestinal sodium transport impairs Cu absorption (Wapnir, 1991). The intestinal concentration of metallothionein is lower in rat pups than in adult animals (Varada *et al*, 1993). Therefore, metallothionein is unlikely to be responsible for increased retention of Cu in the pup's intestine. In adult animals, however, intestinal metallothionein appears to reduce absorption when Cu exposure is continuously higher (Hall *et al*, 1979). According to investigations with stable isotopes, Cu absorption decreases with increasing supply levels. Increasing the supply from 0.8 to 7.5 mg Cu/day will no more than double the absorbed Cu quantity. At high supply levels, Cu whole body retention decreases to 12% while its maximum is estimated to be 63–67%. Absorption, thus, seems to be homeostatically controlled with a set point rate of 0.8–1.0 mg Cu/24 hours (Turnlund, 1991). Little is known about intestinal transport mechanisms for Cu and their regulation. Na/K-ATPase is involved in the transfer of Cu from the enterocytes into the blood.

The bioavailability of Cu depends largely upon interactions with other dietary components. Thus, balance studies in full-term breast-fed babies indicate a Cu bioavailability of 75–77% (Dörner *et al*, 1989). In contrast, Cu absorption from fortified infant formulas based on cow's milk amounted to only 23% (Lönnerdal, 1998), ie it was three times lower. In human breast milk 75% of Cu is bound to soluble milk proteins (Fransson & Lönnerdal, 1983), whereas in cow's milk 75–80% of Cu are bound to insoluble casein (Lönnerdal *et al*, 1985), which is not completely hydrolysed by the infant's immature digestive systems. This is why the difference in Cu absorption between cow's milk and breast milk is particularly marked in preterm infants.

Adequate gastric HCl production facilitates peptic digestion in the stomach and, hence, the availability of Cu in the small intestine. Alkaline pH values in the intestine reduce Cu-bioavailability by formation of Cu-hydroxides and Cu-complexes with a low dissociation constant. Phytates do not impair the availability of Cu to the same extent as that of Fe or Zn (Turnlund *et al*, 1985). Dephytinization impairs Cu bioavailability indirectly: Zn and Fe are less well bound in the lumen and may reduce Cu absorption (Morris *et al*, 1988). The impairment of Cu-absorption by Zn is used therapeutically in Wilson's disease.

Glucose polymers increase the co-transport of Cu with water (solvent drag) and, thus, Cu absorption. Correspondingly, substitution of glucose by fructose reduces Cu bioavailability. A reduced energy metabolism may add to this

effect (Wapnir & Devas, 1995). Large concentrations of amino acids and peptides may bind Cu in the lumen and reduce its absorption. In moderate concentrations, however, such ligands may reduce Cu-hydroxide formation and increase Cu absorption. Organic acids such as citrate, lactate or malate increase the solubility and absorption of Cu (Sable-Amplis *et al*, 1987; Table 2).

3. How is Cu homeostasis maintained?

> 'In the blood Cu is predominantly present in the plasma, where it is bound to albumin and ceruloplasmin. Little is known about Cu plasma binding during transport from the intestine to the liver and its regulation. Hepatic Cu uptake seems to vary extensively in response to changes in Cu supply. The form in which Cu is bound when it reaches the peripheral tissues is discussed controversially. Cu uptake into extrahepatic tissues and fetal tissues is incompletely understood. Chaperones are specific transport proteins which are involved in intracellular Cu transport. The liver regulates Cu elimination via biliary excretion. Cu reabsorption after biliary excretion is small.'

Cu homeostasis requires a delicate balance between Cu absorption, distribution, storage, and elimination. Specific transport proteins in small intestinal enterocytes control intestinal Cu absorption, ie its uptake and transfer from the diet into the blood (cf. Section 2). Absorbed Cu reaches the liver via the portal circulation predominantly bound to transcuprein (Weiss & Linder, 1985), histidine and to the high affinity binding sites of albumin (Masuoka *et al*, 1993). The uptake of Cu by hepatocytes is incompletely understood. A specific Cu transport protein, called hCtr1, appears to play a major part in this process (Pena *et al*, 1999). In the hepatocyte, monovalent Cu is bound to low molecular weight proteins in the cytosol, so-called chaperones. Each chaperone has specific tasks regarding the transport of Cu into cell organelles and regarding the incorporation of Cu into enzymes (Lin & Culotta, 1995). Some of the Cu is stored by binding to cytosolic metallothionein. The metallothionein concentration, and hence its binding capacity for Cu, increases with an increasing Cu supply (Bremner, 1987).

Cu export from the hepatocytes is mediated by incorporation of the metal into ceruloplasmin via a Cu-binding ATP-ase located in the Golgi apparatus (Nagano *et al*, 1998) to mediate its excretion via the bile. At adequate Cu supply levels, a constant amount of Cu is incorported into hepatic ceruloplamin. In copper deficiency CU incorporation into ceruloplasmin is reduced, although its synthesis remains largely unaffected (Nakamura *et al*, 1995). Ceruloplasmin-bound Cu is transported with the plasma to the different tissues. Inhibition of ceruloplasmin synthesis by cyclohexamide has a significant effect on Cu distribution between the organs. However, there is no doubt that organs other than liver and kidney can take up Cu without ceruloplasmin mediation (Lee *et al*, 1993), although to a substantially reduced extend. Though ceruloplasmin excretion controls hepatic Cu export to some extent, it does not affect the amount of Cu in the body. Thus, the role of this protein for Cu homeostasis is presently questioned in favour of its function as ferroxidase (Gittlin, 1998).

Excess Cu is extracted via the bile. This process comprises Cu exocytosis via lysosomes and via a Cu-binding ATP-ase in the canalicular membrane. Besides, an ATP-independent and GSH-dependent Cu transport process is under discussion for biliary Cu excretion (Dijkstra *et al*, 1996). Only marginal amounts of Cu are reabsorbed after biliary excretion (Linder, 1991).

Table 2 Promotion and inhibition of intestinal Cu absorption

Promotion
Adequate gastric digestion
Moderate luminal aminoacid and peptide concentrations (reduced Cu-hydroxide formation)
Organic acids (increased Cu solubility)
Inhibition
Lack of Na and glucose in the intestinal lumen (reduced solvent drag)
High intestinal metallothionein levels (presumably only in adults)
Alkaline pH in intestinal lumen
Phytates (small impact)
Large luminal concentrations of amino acids and peptides (high Cu-binding in the lumen)

4. Biomarkers, diagnostic parameters of Cu status (plasma concentration, Cu in red blood cells, enzymes, ceruloplasmin), diagnostic costs, when is intensive investigation indicated?

> 'The best indicator for Cu status is the hepatic Cu concentration (biopsy). SOD concentration in the erythrocyte can be used to assess Cu deficiency. The Cu concentration in the plasma is an inert indicator due to homeostatic regulation. Low plasma concentrations and SOD activity in erythrocytes mark advanced Cu deficiency states. They do not reveal marginal deficiencies. Because of their limited discriminative power, the methods used to characterise Cu status cannot be considered as adequate.'

The classic approach to determine the quality of a biomarker is to monitor its response to dietary depletion and repletion. Studies in dogs, pigs, cattle, sheep and rats showed decreases in serum/plasma Cu concentrations, in the hepatic Cu content, in plasma ceruloplasmin concentrations, in the SOD activity in liver or erythrocytes, or in the activity of cytochrome-C-oxidase in the liver and duodenal mucosa during Cu-deficient feeding (Baker & Ammerman, 1995). A decreasing dietary Cu intake correlated best with the hepatic Cu content, followed by hepatic SOD activity. Increasing dietary

supply levels correlated best with the hepatic Cu content. However, the relationship between dietary Cu content and the Cu hepatic concentration is strongly species-specific (Baker & Ammerman, 1995).

Depletion and repletion studies in healthy volunteers help to assess the value of the different diagnostic parameters: (1) the concentration of Cu and ceruloplasmin in the plasma remained unaltered, when they were challenged by a daily supply of 0.57 mg Cu for 105 days. The SOD activity in erythrocytes and the cytochrome-C oxidase activity in the platelets, however, was decreased. Administration of 2 mg Cu for 35 days increased the activity of cytochrome-C-oxidase and glutathione peroxidase (Milne & Nielsen, 1996). (2) The plasma concentrations of Cu and ceruloplasmin decreased following a dietary challenge with 0.38 mg Cu for 42 days. A 24 day repletion with 2.5 mg Cu/day led to no significant increase of these parameters (Kelley *et al*, 1995). (3) After dietary Cu depletion (0.79 mg Cu/day for 24 h) and subsequent Cu repletion (7.53 mg Cu/day for 42 and 24 days), no changes in plasma Cu and ceruloplasmin concentrations or in erythrocyte's SOD activity were observed (Turnlund *et al*, 1990). (4) After reduced Cu intake for 77 days (1.03 mg/day 2850 kcal with 20% fructose), the plasma concentrations of Cu and ceruloplasmin were unchanged, while erythrocyte's SOD activity decreased and increased again after repletion (3 mg Cu/day for 21 days, Reiser *et al*, 1985). The serum-Cu concentration is homeostatically regulated. This parameter shows circadian changes with a peak in the morning, which is higher in women than in men, and increases with age. Oestrogen intake, pregnancy, infections, inflammation and stress increase plasma Cu concentration while corticosteroids and corticotrophin administration decrease it (Johnson *et al*, 1992; Milne & Johnson, 1983). Ceruloplasmin in the plasma is regarded as an acute phase protein (Milne, 1994). It is influenced in analogy to plasma Cu concentrations. The activity of Cu/Zn-SOD in erythrocytes, in contrast, is not affected by such influences. It is reduced in Cu depletion and increases again on repletion and, therefore, appears as a useful marker of Cu status (Johnson *et al*, 1992). However, because of the limited sensitivity of all of these parameters, no optimal method is available to assess marginal Cu deficiency.

It is common practice to determine Cu concentration in serum by electrothermal atomic absorption spectrometry. Standard indications are the diagnosis of Wilson's disease, Menkes' syndrome, nutritional Cu deficiency or Cu intoxication. The Cu content in tissues or specific blood cells is not routinely investigated. Such analysis is time-consuming, difficult to standardize and tissue sampling is an invasive process. SOD activity is also not measured routinely. There are guideline values for Cu concentrations in plasma and erythrocyte SOD activity which, however, are not generally accepted reference values.

A promising new parameter for the determination of excess Cu intake is an elevated serum diamine oxidase activity (DAO; Kehoe *et al.*, 2000). Evans *et al* (1998) suggested determining free plasma Cu concentrations directly, instead of calculating it from other parameters. A suitable method was developed by Gutteridge for other purposes (Gutteridge, 1984). The Cu-specific parameter measured colorimetrically in this case is the extent of oxygen radical formation during Cu-dependent DNA oxidation.

5. What is the role of Cu in oxidative stress?

> 'Cu-overload favours the development of oxidative stress. Cu deficiency has also been proposed as a possible cause of oxidative stress, though this has not been established with certainty. There are, however, individual results to support this suggestion.'

In situations of oxidative stress newly formed oxygen radicals cannot be adequately detoxified by antioxidants and excess free radicals cause cellular damage. Hydroxyl radicals are formed *in vivo* mainly by reaction with H_2O_2, in accordance with eqn (1):

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + {}^{\bullet}OH + OH^- \qquad (1)$$

Although the metal ion M^{n+} can stand for Ti^{3+}, Fe^{2+}, Cu^+, Co^{2+} as well as for chromium, manganese, vanadium and nickel complexes, only Fe^{2+} and Cu^+ seem to be capable to catalyse Fenton chemistry *in vivo* (Gutteridge, 1994; Wiedau-Pazos *et al*, 1996). The rate for this second order reaction is as low as $10^2\ M^{-1}\ s^{-1}$. If one considers that the reaction partners H_2O_2, Fe^{2+} and Cu^+ are present at a concentration of about 1 μM under physiological conditions, the assumed reaction rates for Fe^{2+} and Cu^+ can be compared. If a cell volume of 10^{-12} L is assumed and a second order reaction constant of $k = 76\,M^{-1}\,s^{-1}$, about 46 molecules of $^{\bullet}OH$ are formed per cell per second (Halliwell & Gutteridge, 1990) when Fe^{2+} is used as the catalyst (see eqn (2)).

$$N = 7.6 \times 10^{-11} \times 10^{-12} \times 6023 \times 10^{23} \qquad (2)$$

In contrast, the turnover rate with Cu^+ as a catalyst is $4.7 \times 10^3\,M^{-1}\,s^{-1}$. This is orders of magnitude greater than with Fe^{2+} as a catalyst and results in the production of 2840 $^{\bullet}OH$ per cell per second. It must be considered here that Cu^{3+} may be produced in addition to or instead of radicals.

Cu^+ and Cu^{3+} can be produced by the reaction with the superoxide anions (eqns (3) and (4)).

$$Cu^{2+} + O_2^- \rightarrow Cu^+ + O_2 \qquad (3)$$

$$Cu^{2+} + O_2^- + 2H \rightarrow Cu^{3+} + H_2O_2 \qquad (4)$$

However, it is not certain whether metal ions undergo reactions with superoxide anions *in vivo* and, hence can produce oxidative stress.

Cu deficiency is also discussed to produce oxidative stress, which was studied in different model systems. Assuming that the activity of antioxidative enzymes and the extent of oxidative modification of erythrocyte proteins are reliable indicators for oxidative stress, rats were fed a Cu-deficient

diet. While hepatic Cu concentrations decreased by about 80% during Cu-deficient feeding, the Cu content in nuclei of hepatocytes did not change (Lai *et al*, 1996). The transcription rates of the enzymes Cu,Zn-SOD, glutathione peroxidase and glycerine aldehyde-3-phosphate-dehydrogenase remained unaltered, those of Mn-SOD and of beta-actin increased and the transcription rate of catalase decreased in Cu deficiency (Lai *et al*, 1996). The activity of cytoplasmic Cu,Zn-SOD in erythrocytes decreased as well, white the amount of carbonylated subunits of spectrin, an erythrocyte membrane protein (Sukalski *et al*, 1997) increased significantly. these examples show that experimentally induced Cu deficiency in rate influences the activity of hepatic antioxidative enzymes differentially. The activity of Cu,Zn-SOD is reduced, although the rate of transcription remains unchanged. Thus, presumably, the ratio between apoenzyme to enzyme will increase in situations of inadequate Cu supply. This finding underlines the importance of Cu,Zn-SOD as an indicator for Cu deficiency.

6. Which functional deficits are observed in Cu-deficiency?

> 'Clinical manifestations of Cu deficiency are very rare and are predominantly the results of a dietary Cu deficiency. Among the clinical manifestations of Cu deficiency are anaemia, neutropenia and increased bone fragility. An impact of Cu deficiency on the immune system has been suggested.'

Cu uptake with the diet is essential for man and animals and generally exceeds the minimum requirement of this trace element. Experimental Cu deficiency in animals may cause cardiac and vascular disease, which can partly be explained by a reduced activity of Cu-dependent enzymes, Neurological deficits were observed in Cu-deficient sheep (Williams, 1983), thromboses (Klevay, 1985) and reduced sperm motility and fertility in rats (Battersby & Chandler, 1977). In children Cu deficiency produced hypochromic anaemia and disturbed the maturation of myeloid cells in the bone marrow (Cordano, 1998). The erythrocyte's half-life was reduced, due to altered fluidity and increased sensitivity of the membrane to lipid peroxidation (Rock *et al*, 1995). Accordingly, one of the early clinical signs in Cu deficiency is neutropenia. Neutropenia has been related to an increased frequency of respiratory infections in Cu deficiency. Besides, the number of these cells in the bone marrow is reduced in Cu deficiency, which may in part be attributed to a reduced survival of neutrophilic granulocytes. The skeleton tends to be osteoporotic in Cu deficiency, with an increased risk of fractures. Erosion and reactive spur formation are found in the metaphysis (Cordano, 1998).

Therefore, Cu deficiency is an unlikely cause of teratogenic effects in men, although neural tube defects (Morton *et al*, 1976) and an increased risk of anencephaly (Buamak *et al*, 1984) have been associated with Cu deficiency. The importance of Cu for foetal development becomes obvious in animal deficiency symptoms. A spastic paralysis of the hind legs in lambs is associated with hypomyelinization in the brain in severe Cu deficiency (Hurley & Keen, 1979). This syndrome is called 'sway back disease'. It is through to be caused by deficiency in Cu-dependent cytochrome-C-oxidase (Mills & Williams, 1962). Deficient elastin incorporation in lung tissue (Abdel Mageed *et al*, 1994) and blood vessels and a defective collagen matrix in dogs and pigs (Hurley & Keen, 1979) appear to be the consequence of a reduced activity of Cu-dependent lysyl-oxidase.

7a. Is the recommended dietary Cu intake reached in the average population?

> 'The daily Cu intake in adults should reach 20 μg/kg body weight; for infants 50 μg Cu/kg body weight should be reached. Regarding the compliance with nutritional advice, Cu has to be counted among the critical elements.'

The DGE recommends an intake of 1.0–1.5 mg Cu/day for adolescents and adults. The corresponding recommendation of the Scientific Committee on Food of the European Commission is 1.1 mg Cu/day for adults (Scientific Committee on Food, 1994). The DEG assessment was based on dietary balance studies, from which intakes of 75 μg Cu/kg in infancy, 50 μg/kg in older infants, 40 μg Cu/kg in children aged 7–10 y of age, and finally 20 μg Cu/kg in adults were derived (Deutsche Gesellschaft für Ernährung, 1991; National Research Council, 1989). In addition to the recommended dietary Cu intake, which is required to maintain normal plasma levels, the WHO derived an upper safe level for Cu in food (12 mg/day for male and 10 mg/day for female adults on the basis on the basis of 180 μg/kg body weight and day) which should not be exceeded (WHO, 1996).

Daily Cu intake was studied in Austria using a 7 day weighing protocol (König & Elmadfa, 1993; Elmadfa *et al*, 1999). Data on Cu concentrations in plasma were also available for part of this group. The data show that a Cu intake according to current DGE recommendations was not sufficient in all cases to keep plasma Cu concentrations in the normal range for children, adolescents and elderly (0.8–1.2 mg/l; König & Elmadfa, 1993; Elmadfa *et al*, 1999). According to the 1994–1996 Continuing Survey of Food Intakes by Individuals, Cu intake in individuals over 60 y of age in the US was 1.3 ± 0.7 mg Cu/day for men and 1.0 ± 0.5 mg Cu/day for women which is less than recommended. Main dietary Cu sources were legume, potato and potato products, nuts, seeds and beef (Ma & Betts, 2000). Also, chocolate foods can be a good source of Cu (Joo & Betts, 1996). Correspondingly, the mean Cu intake in various European countries was below the recommended minimum intake of 1.2 mg Cu/day in adult males in about 10% of

subjects under investigation and below the minimum normative population intake in about 25% (WHO, 1996; 1998). In spite of these findings, according to the WHO, Cu deficiency is relatively rare in humans under normal physiological conditions. Clinical signs of deficiency are only observed under exceptional circumstances (Underwood, 1977), eg in children with 'cow's milk anaemia', during rehabilitation after malnutrition or when formula milks are inadequately fortified with Cu, in adults with protein-energy malnutrition, in enteropathies and during total parenteral nutrition as a consequence of inadequate Cu supplementation of infusion solutions (WHO, 1998; see Section 7b).

7b. Are there special groups at risk for inadequate Cu supply?

'Deficiency due to increased requirements: disease states, pregnancy (last trimester), premature infants, periods of rapid growth, parenteral nutrition. Deficiency due to inadequate intake: eg maldigestion/malabsorption, reducing diets.'

Deficiency due to increased requirements

Cu deficiency states in children with massive diarrhoea in developing countries (Castillo-Duran *et al*, 1990; Sachdev *et al*, 1989), especially in association with growth spurts after protein-energy malnutrition seem to be the most common cause of Cu deficiency (Castillo-Duran & Uauy, 1988). The loss of Cu in children with diarrhoea was twice that in controls (Castillo-Duran *et al*, 1988). Consequently, plasma levels of Cu were reduced by half (Rodriguez *et al*, 1985). Also concomitant administration of zinc, fructose or chelators increase the demand and appear to cause Cu deficiency more frequently than inadequate dietary Cu supply (Keen *et al*, 1998). In nephrotic syndrome (Pedraza-Chaveri *et al*, 1994) and in patients with severe burns (Shakespeare, 1982), excessive loss of Cu may occur and the Cu requirement is increased. Other conditions of this type are short bowel syndrome, cystic fibrosis, coeliac disease, tropical and non-tropical sprue, intestinal fistulas and following gastrectomy and ileo-jejunal bypass operations (Beshgetoor & Hambidge, 1998).

During the second half of pregnancy the foetus acquires about 50 μg Cu/kg/day (Widdowson *et al*, 1974). Correspondingly, pregnant women reduce biliary Cu excretion by hormonal adjustment (McArdle, 1995) and serum Cu concentration increases by a factor of about 2 until the final trimester (Keen *et al*, 1998). Therefore, Cu deficiency in pregnancy is rare. Premature babies have an increased risk of Cu deficiency. *In utero* the foetus stores Cu predominantly during the final; trimester. A mature infant has acquired Cu stores of approximately, 15 – 17 mg Cu during pregnancy, 2.5 – 9.0 mg of which are found in the liver (Widdowson *et al*, 1974). These reserves prevent Cu deficiency in the first 47 months after full-term birth (Cordano, 1998). If delivery becomes due before the 34th week of pregnancy, plasma Cu concentrations are decreased correspondingly (Sann *et al*, 1980). Cu mobilisation from hepatic stores depends on liver maturation (Mason *et al*, 1981). This is why Cu supply to organs and enzymes does not reach the same level in premature as in full-term babies before the same age after conception is reached (Hillman, 1981).

The question whether abundant Cu stores and ineffective biliary Cu excretion in neonates presents an evolutionary advantage or a toxicological risk (Danks, 1991) (see Section 8), thus, has different answers in premature babies and full-term infants. Due to rapid growth, premature babies have an increased Cu requirement (Shaw, 1992) as their digestive capacity and intestinal Cu absorption is not yet fully matured. Therefore, about a third of very low birth weight infants fed with normal infant formula have a suboptimal Cu status at the age of 6 – 12 months (Beshgetoor & Hambridge, 1998). The American Academy of Paediatrics recommends to feeding infant formulas with a Cu content of 900 μg Cu/l to premature babies to compensate for these deficits. This recommendation seems to be justified because these children absorb no more than approximatly 15% of the Cu supplied with bottled milk due to its high casein content (cf. Section 2), whereas they absorb about 60% from breast milk (Ehrenkranz *et al*, 1989).

Deficiency due to inadequate intake

Malabsorption in the context of chronic enteropathies may result in hypocupraemia (Cordano & Graham, 1966). Several case reports have described Cu deficiency states in association with coeliac disease (Goyers *et al*, 1985). Total parenteral nutrition without adequate Cu supplementation can lead to overt Cu deficiency not only during rapid growth (Shulman, 1989), but also in adults (Fleming, 1989; Okada, 1994). Accordingly, the American Medical Association has recommended guaranteeing a supply of 0.5 – 1.5 mg Cu/day during total parenteral nutrition (American Medical Association, 1979). This is based on several observations: A supply of 1.3 mg Cu/day prevented Cu deficiency in adults (Shenkin *et al*, 1987). As little as 0.3 mg Cu/day maintained an adequate Cu status in adults in another study (Shike *et al*, 1981). Cu deficiency was compensated by administration of 1 mg Cu/day over a period of 2 weeks, followed by 0.5 mg Cu/day in adults and 0.2 mg Cu/day in children to maintain the status (Shike *et al*, 1981; Shike, 1984). However in cholestasis, the parenteral Cu supply must be reduced (WHO, 1998).

8. How can damage caused by Cu overload be diagnosed? Are there risk groups for oral Cu overload?

'Clinical diagnosis of Cu overload can only be established with certainty by liver biopsy. There is controversy as to

whether Cu poses a special risk for infants and small children that are not breast-fed.'

An infant of 4 kg body weight fed with reconstituted dried milk has an intake of up to 140 ml/kg/day. At a weight of 10–20 kg this is reduced to about 100 ml/kg/day, while an adult of 70 kg body weight consumes about 30 ml of fluid/kg/day. Unlike in bottle-fed babies, adults fluid intake derives from various sources which helps to avoid one-sided exposure. Due to these reasons, the exposure of infants and small children to potentially toxic substances in the drinking water is 3–5 times higher than for adults. This is no problem as long as dose–response relationships are assessed sufficiently conservative (Dieter, 1995). However, our knowledge on the dose-response relationship for hazardous Cu effects is incomplete.

Minimum intake *vs* maximum intake of Cu

A daily dietary intake of 1–2 mg Cu/day seems necessary to provide for Cu-dependent metabolic processes in adults. This corresponds to the most recent lower limit of the 'acceptable range of oral intake' (AROI) for adults of 20 g Cu/kg/day (WHO, 1998). Due to the increased metabolic requirement, the corresponding value for infants is 50 μg Cu/kg/day (=0.2–0.3 mg Cu/day; cf. Section 7a). On the other hand, the WHO (1998) stated that the upper limit of the AROI for adults is not known; it is probably in the order of several, but not many, mg per day per adult. There is no statement regarding the upper limit of AROI in infants or to whether the 'upper limit of the AROI' is a threshold for adverse Cu effects or rather a maximum for a physiologically desirable Cu intake (WHO, 1998). Some calculations may help to differentiate between these two options.

Physiologically desirable Cu concentrations in the drinking water for bottle-fed babies

The Cu content in 75 different commercial milk formulas from 10 manufacturers varies between 400 and 700 μg/l (mean *ca* 500 μg/l), when the milk was rehydrated with Cu-free drinking water (Blasco *et al*, 1999). The corresponding Cu intake of bottle-fed babies is up to 75 μg/kg/day, which is 1.5 times the minimum intake recommended by the WHO (1998). Additional Cu intake from the water, thus is not needed from a physiological point of view. The upper limit of the excretory capacity in infants is estimated to be 50 μg Cu/kg/day (Aggett, 1999). Taking this value and assuming 50% absorption for Cu in the gastrointestinal tract, no more than 0.17 mg Cu/l in the milk are needed to satisfy physiological requirements. However, the preliminary WHO drinking water standard (2 mg Cu/l, WHO, 1993) is more than 10 times higher. This value derives from data on the acute gastrointestinal side effects of Cu that were characterised as not very solid. It was noted that studies are underway to quantify Cu-induced effects in man more exactly, especially in sensitive subgroups (WHO, 1993). Due to this situation it was discussed that exposure to milk containing 2 mg Cu/l, as approved by the WHO (1993) and possibly also to lower Cu concentrations might be hazardous for infants regarding the development of liver cirrhosis (Hadorn, 1999). On the other hand, a single case of liver cirrhosis was proven to be associated with public water supply, although millions of German homes are equipped with Cu plumbing (Vergara *et al*, 2000). Still, the scarcity of positive observations does not rule out completely that Cu in the drinking water may increase the hazard for hepatic damage in infants (Dieter *et al*, 1999).

How much Cu have infants with 'idiopathic copper toxicosis (ICT)' ingested with resuspended formula milk?

The idea that Cu concentrations between 0.2 and 2.0 mg/l may be harmful to the health of bottle-fed babies seems unlikely on the basis of plausible pharmacokinetic considerations (Tanner, 1998): assumed that the liver of a 1-y-old child suffering from ICT weighs 900 g (for comparison, a normal, healthy liver would weigh about 300–400 g) and has the average pathological content of *ca* 400 μg Cu/g wet weight, the total liver Cu content would be 360 mg Cu. Up to 9 mg of Cu are acquired by the liver during pregnancy (see Section 7b) and are likely to be excreted or redistributed during the first year of life. If the child is assumed to consume 1 of formula milk containing 3 mg Cu/l over a year, which is a high estimate, and if absorption amounted to 50%, total Cu absorption corresponds to 550 mg Cu. Thus, the liver would store over 70% of the absorbed Cu quantity without providing for Cu excretion. However, a healthy child excretes *ca* 30 μg Cu/kg/day, corresponding to 100–200 μg/day, which sums up to at least 50 mg during the first year of life (Aggett, 1999).

During the perinatal period, the liver contains 50–60% of the body Cu content at most. From the 6th month on this fraction decreases to 10–20%, unless a genetic disorder of Cu metabolism were present. Taking these figures, the daily absorption of 1.5 mg Cu/day from the diet would correspond to a hepatic Cu content of 60–70 mg Cu at the end of the first year of life. This corresponds to a Cu concentration of about 200 μg/g wet weight in a normal-weight liver (300–400 g) and to just 70 μg/g wet weight in an enlarged liver of 900 g. However, hepatic Cu concentrations determined in ICT children are 20–30 times higher than that! Assuming an intake of 3 mg Cu/day with reconstituted milk, such high hepatic Cu concentrations can accumulate only if biliary Cu excretion were far below the normal rate (30 μg/kg/day) and if much less than 80% of the body Cu content were distributed to other organs after the 6th month of life, ie when Cu metabolism were markedly disturbed.

Remaining uncertainties

Ceruloplasmin synthesis and, thus, Cu distribution to other tissues is underdeveloped during the first 3–6 months of life.

Between the first and fourth month the mean plasma concentration of ceruloplasmin increases from barely 1 to 2 μmol/l, and reaches adult levels (2–3 μmol/l) by the 12th month of life. This development was observed in breast-fed infants as well as after intake of formula milk with low Cu and high Cu content (85 *vs* 500 μg Cu/l Cu). The Cu content in the supplementary diet was not determined in this study (Salmenperä *et al*, 1989).

Biliary Cu excretion increased in parallel to the increase in ceruloplasmin synthesis, reaching adult levels during the 3rd to 6th months. Thus, biliary excretion is as ineffective as ceruloplasmin synthesis in early infancy. In addition, the frail bile ducts in infants are particularly susceptible to infection. 'Neonatal hepatitis' may impair or stop the bile flow and lead to cholestatic Cu retention (Epstein, 1983; Danks, 1991). For these reasons, normal hepatic Cu concentrations found in foetuses and during the first 6 months of life (200–400 μg/g dry weight) are about 10 times higher than in adults (Aggett, 1999). The assumption that such high Cu levels are essential stores for physiological requirement in full-term neonates is difficult to maintain (Danks, 1991), as the Cu amount supplied with the breast milk under normal conditions (0.1–0.3 mg/l) is easily adequate for its essential requirements (Aggett, 1999; Salmenperä *et al*, 1989).

It cannot be decided at present whether the high hepatic Cu levels in infants indicate an increased sensitivity for Cu exposure. If one compares the example of an adult who developed severe cirrhosis after 3 y exposure to 0.5–1.0 mg Cu/kg/day (O'Donohue *et al*, 1993, to that physiological Cu intake during the first 6 months of life (0.4–0.4 mg Cu/kg/day) one feels tempted to speculate that a healthy infants might be less sensitive to high Cu exposure than the adult liver, in spit of its underdeveloped Cu excretion capacity. Correspondingly, a prospective exposure study involving 128 infants than 3 months (Olivares *et al*, 1998) and the analysis of copper exposure in 1178 children older than 9 months of age (Pettersson & Rasmussen, 1999) did not indicate any particular sensitivity to Cu in those children. Despite daily administration of 2 mg Cu/L (Olivares *et al*, 1998) or an estimated load of up to 3.2 mg Cu/day with the drinking water (Pettersson & Rasmussen, 1999) no signs of liver damage were observed in the exposed children. However, none of these studies included the critical age group of infants younger than 3 months, who might be particularly sensitive to Cu.

9. Is there a relationship between Cu metabolism and (a) neurodegenerative diseases and (b) cardiovascular diseases?

'Findings in the field of molecular biology suggest an association between Cu metabolism and neurodegenerative diseases. Also, there are mechanisms that suggest a relationship between Cu intake and coronary heart disease (CHD).'

Neurodegenerative diseases

Neurodegenerative diseases appear to be directly related to Cu metabolism. Menkes' syndrome and Wilson's disease are inherited disorders with a functional disturbance of two membrane-located ATP-ases for the transport of Cu ions (Bull *et al*, 1993; Chelly & Monaco, 1993). In Wilson's disease Cu excretion via the bile ducts is disturbed. Consequently, Cu accumulates predominantly in the liver. In Menkes' syndrome, on the other hand, Cu-dependent enzymes, such as cytochrome-C oxidase and lysyloxidase, are not adequately supplied with Cu.

In Menkes' syndrome, clinical and pathological features are already present at birth, implying that Cu-dependent enzymes are of importance already in the prenatal phases of CNS growth and development. Myelination disorders of nerve cells, diffuse atrophy of the brain, focal disintegration of the grey matter with destruction of axons in the white matter and extensive necrosis of Purkinje cells in the cerebellum are observed in Menkes' syndrome. These are sequels of inadequate Cu supply to Cu-containing enzymes and lead to mental retardation of the patients.

Hepatic Cu deposits in Wilson's disease damage the liver. In adolescents and young adults an increased number of neurological deficits show up. Cu deposition in the basal ganglia leads to gliosis and to neurological deficits with symptoms of Parkinson's disease. They go along with changes in personality, depression and schizophrenia, although the molecular mechanisms of these events are not known.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with a sporadic and an inherited form. A loss of motor neurones in the spinal cord and in the brain causes progressive muscular weakness. Observations in siblings have shown that mutations in Cu, Zn-superoxide dismutase (SOD1) may be responsible for the disease (Deng *et al*, 1993; Rosen *et al*, 1993). Animal experiments showed that the disease is not due to decreased enzyme activity. The mutant SOD1 enzyme can also act as a peroxidase as a result of a change in protein conformation (Matzuk *et al*, 1998; Reaume *et al*, 1996). In the presence of Cu the peroxidase activity increases free radical activity, which can be demonstrated by indirect methods in transgenic animals and in patients (Liu *et al*, 1998; Ferrante *et al*, 1997). As cofactor for the mutant SOD-1 molecules, Cu is likely to be directly responsible for the toxic effects. The radicals seem to oxidise aggregate proteins. These changes may retard axonal transport and damage the neurofilaments of motor neurones (Brujin *et al*, 1998; Williamson & Cleveland, 1999).

Alzheimer's disease is a progressive neurodegenerative disease. It is regarded the most common type of dementia. Its particular characteristic is a deposition of amyloid-Aδ-proteins which are observed in all sporadic and familial

forms of the disease (Selkoe, 1998). There is direct evidence that oxidative stress is involved in Alzheimer pathogenesis. Thus, increase oxidation of proteins and DNA is seen in those brain regions that show amyloid deposits first (Markesbery & Carney, 1999). Cu ions can stimulate the aggregation of synthetic Aβ-protein *in vitro*. The concentration is increased 6–8-fold in the characteristic protein deposits in affected patients (Atwood *et al*, 1998; Lovell *et al*, 1998). This fits in with the finding that amyloid-Aδ deposits can be redissolved in the presence of zinc- or Cu-specific chelators in the brain of Alzheimer patients post mortem (Cherny *et al*, 1999). A probable source for the Cu that concentrates in the deposits is the amyloid precursor protein (APP). It contains a Cu-binding domain from which amyloid-Aδ is naturally formed by proteolytic activity (Selkoe, 1998; Multhaup *et al*, 1997).

Numerous findings support the hypothesis that APP is involved in the transport of Cu into the cell. APP is synthesised as a membrane-located and secreted protein. It binds Cu and can reduce Cu^{2+} to Cu^{+} (Hesse *et al*, 1994; Multhaup, 1997; Multhaup *et al*, 1996). The protein is transported from the cell to the axonal membrane and to the dendritic plasma membrane and could, thus, transport Cu^{+} along this way (Simons *et al*, 1995). Cu^{+} bound to this protein is rapidly oxidized by reactive oxygen species (Multhaup *et al*, 1999). This reaction splits APP. Some fragments contain the entire Aδ domain and are regarded as neurotoxic derivatives of APP within the cell (Simons *et al*, 1996). By virtue of their Cu-binding domains, APP–Cu complexes themselves can cause oxidative stress in neuronal cell culture systems (White *et al*, 1999). The most recent evidence for a role of APP in cellular Cu homeostasis comes from cell and animal models used in Alzheimer's research. In mice that do not produce any APP, Cu concentrations in the liver and cerebral cortex are increased by 80% and 40%, respectively (White *et al*, 1999). Besides, additional Cu in the medium also affects the physiological processing of APP, presumably by regulation of APP transport via a conformational change in the protein (Borchardt *et al*, 1999). Thus, an involvement of Cu in Alzheimer pathogenesis is conceivable, eg when the transport of APP–Cu complexes were disturbed or when APP molecules would aggregate in axons and thereby initiate the amyloid cascade.

Prion diseases generally occur as sporadic, dominantly inherited or infective diseases (Aguzzi & Brandner, 1999). Creutzfeldt–Jacob disease (CJD) in man, for example, is characterised by a rapidly progressing dementia and cerebellar ataxia. Spongiform changes are found in the tissue with gliosis of the astrocytes. In the course of pathogenesis, the cellular prion protein PrP^{c} is transformed into an infectious prion protein PrP^{Sc} and aggregates in the form of amyloid-like plaques in affected brains (Merz *et al*, 1981).

There are reasons to believe that cellular PrP is involved in CNS Cu homeostasis. Thus, the Cu chelating agent cuprizone induces similar neurological alterations in mice as were observed in prion diseases (Kimberlin *et al*, 1974; Pattison *et al*, 1971). In contrast to APP-deficient mice (see above), the brain cells of mice in which the PrP gene is turned off are more sensitive to Cu salts (Bueler *et al*, 1992). They show a reduced SOD1 activity and a lower Cu content in the cell membranes (Brown *et al*, 1997; 1998). Since Cu ions stimulate PrP uptake into cells (Pauly & Harris, 1998), it is assumed that PrP might be involved in cellular uptake.

Cu binds to the N-terminus of PrP (Hornshaw *et al*, 1995; Muira *et al*, 1996) and changes the conformation of the protein into a δ-folding structure (Stöckel *et al*, 1998). *In vitro*, Cu binding causes the prion protein to regain its infective potential and its resistance to proteolytic destruction (McKenzie *et al*, 1998). PrP^{Sc} molecules are found in the affected tissues as protein/metal ion complexes containing zinc and Cu. Obviously, Cu binding causes conformational changes in PrP^{Sc} which could also explain the diversity of prion strains (Wadsworth *et al*, 1999).

The physiological significance of Cu binding to PrP is not yet elucidated. However, on the basis of the findings described above one can assume that PrP co-regulates cellular Cu homeostasis. It is involved in cellular Cu uptake and subsequently, as Cu chaperone, it delivers Cu to corresponding target proteins.

Coronary heart disease (CHD)

Disturbed Cu uptake has been proposed as a risk factor for coronary heart disease (Klevay, 1975; 1980). HMG-CoA-reductase is a key enzyme in endogenous cholesterol synthesis and its activity is increased in Cu deficiency (Yount *et al*, 1990). In parallel, the sensitivity of VLDL and LDL to oxidation increases in Cu deficiency (Raysiguier *et al*, 1993). LDL oxidation is a significant pathogenetic factor in the development of arteriosclerosis and hence of CHD. Correspondingly, there is evidence that an inadequate Cu intake favours the development of hypercholesterolaemia. An increase in total cholesterol, LDL-cholesterol and decrease in HDL-cholesterol were observed in Cu deficiency (Klevay *et al*, 1984). The effect of Cu depletion in animal vessels and the changes found in patients with CHD show apparent similarities (elastin destruction, fibrosis, intramural haemorrhages, increase in mucopolysaccharides, necrosis and the proliferation of smooth muscle cells; (Klevay, 1993). The Yi People Study (He *et al*, 1992) found an inverse correlation between serum Cu and the risk of CHD. However, Cu intake was not monitored in this study, and the problems related to the use of serum copper concentrations as the only indicator of Cu status were commented on earlier. In contrast to these observations, some epidemiological studies found a positive correlation between Cu uptake and the risk of CHD (Manthey *et al*, 1981; Kok *et al*, 1988).

Macroangiopathy in Cu-deficient animals is primarily attributable to a decreased activity of lysyloxidase. This Cu-dependent enzyme cross-links collagen and elastin (Reiser *et al*, 1992), and a decreased activity enzyme impairs the integrity of the arterial wall (Schuschke, 1997). Cu deficiency might also impair the effect on the elasticity of the arterial

wall via a reduction in NO-induced vasodilatation (Saari, 1992; Schuschke *et al*, 1992). This effect is supposed to be mediated via increased NO degradation by superoxide anions as a consequence of decreased SOD activity in Cu deficiency (Dubick *et al*, 1988; Belch *et al*, 1989). These points a reviewed in greater detail by Strain (1994).

10. What damage can be caused by Cu overload?

'Acute: gastrointestinal symptoms, death. Chronic: damage to target organs show individual variation.'

Acute toxicity

After acute oral intake Cu is relatively non-toxic for man. Acute intoxications have primarily been observed after accidental or suicidal ingestion, and due to inhalation of Cu dusts and fumes at work. Milligram amounts of soluble Cu salts can be ingested with fruit juices that were stored in Cu containers or with water from Cu plumbing. Such intake may cause vomiting and diarrhoea. In severe cases, soluble Cu compounds in gram quantities lead to haemolysis, liver and kidney damage, coma and death. The lowest acutely fatal dose in man is about 10 g Cu. Inhalation of Cu fumes irritates the upper airways, and may lead to malaise, stomach pain and diarrhoea.

Chronic toxicity

Chronic Cu intoxication from contaminated food is rare and mainly affects the gastrointestinal tract and the liver. In patients with Wilson's disease, haemolysis, neurological symptoms and renal damage are observed in addition. A family in Vermont experienced recurrent gastrointestinal irritation as a result of Cu-contaminated water consumption (2.8 – 7.8 mg Cu/l; Spitalny *et al*, 1984). Excess Cu concentrations are also found in infant milk stored in Cu containers or resuspended in Cu contaminated water (Tanner, 1998; Sethi *et al*, 1993; see Section 8). A 26-y-old man, who had taken 30 mg Cu/day as Cu gluconate for 2 y and then 60 mg Cu for a further year developed cirrhosis of the liver and acute liver failure (O'Donohue *et al*, 1993).

Mechanisms of toxicity

Cu toxicity is linked to its unique electron configuration. Thus, Cu^{+} ions easily be polarised and bind predominantly to ligands rich in nitrogen and sulphur. Cu^{2+} ions tend to form complexes with ligands containing nitrogen and sulphur. Therefore, Cu is comparably reactive in biological matrices and can form strong bonds with a wide variety of structures that are rich in electrons. The replacement of other essential metals in enzymes by Cu or Cu-binding to macromolecules may have deleterious effects on their function and structure, resulting, for example, in enzyme inhibition and alterations of DNA and membrane structures (Alt *et al*, 1990). In addition, Cu is involved in redox reactions. Cu ions are able to catalyse the formation of hydroxyl radicals via Fenton chemistry (Goldstein & Czapsky, 1986; see Section 5). These redox-reactions can increase cytoplasmatic calcium concentrations, cause ATP depletion and excess oxidation of thiols, DNA and membranes, which may finally lead to a loss of cellular integrity (Bremner, 1998).

Apart from whole body Cu content, the subcellular distribution and binding behaviour is of crucial importance for Cu toxicity. Thus, Cu transfer from the cytoplasm into lysosomes appears to be essential for the development of Cu-induced liver damage (Goldfischer & Sternlieb, 1968; Klein *et al*, 1998). Chaperones mediate intracellular Cu distribution to the sites of functional or toxic action (Pena *et al*, 1999). The low-molecular protein metallothionein is crucial for intracellular Cu storage and detoxification. Thus, Cu administration induces metallothionein synthesis *in vivo* (Bremner, 1987) and high metallothionein concentrations reduce Cu toxicity (Freedman *et al*, 1989). Metallothionein is also assumed to have a direct antioxidant effect by reacting readily with hydroxyl radicals (Thornalley & Vasak, 1985). Correspondingly, mouse cells that can't synthesise metallothionein proved to be particularly sensitive to oxidative stress (Liu *et al*, 1995).

11. Future research requirements

'Markers for the determination of Cu status and Cu requirement (SOD); mechanisms of Cu homeostasis at the molecular level; Cu and gene expression; polymorphisms, the regulation of Cu transport at the blood – tissue barrier, molecular mechanisms of age-dependent Cu transport across the blood – brain barrier and of the distribution in blood (fractions); molecular mechanisms of chronic toxicity in relation to the cellular and subcellular Cu distribution.'

The significance of Cu for human nutrition has not been adequately elucidated. In particular, reliable biomarkers are lacking to permit reliable determination and interpretation of the individual Cu status. Research preferences in this field should be on the clinical applicability. The lack of reliable markers for Cu status makes it difficult to assess the benefit of increased Cu intake for risk groups or in the prevention of disease and to determine threshold values for Cu toxicity. From a toxicological point of view, Cu metabolism in the neonate needs to be investigated in detail, particularly absorption and hepatic retention and excretion of Cu. Therefore, a better understanding of the mechanisms of Cu homeostasis would be helpful, eg the dependence of Cu transport across the blood – brain barrier on concentration and age. Using molecular methods it should be possible to study the effects of marginal Cu intake on the gene expression of Cu-

dependent transcription factors. A particular challenge for the future is the demonstration of genetic polymorphisms in the expression of Cu-dependent proteins and their functional consequences. With such data it will it be possible to define risk groups for excessive or inadequate Cu intake and prophylactic treatment.

Note

The 15th Hohenheim Consensus Workshop took place in March 1999 at the University of Stuttgart-Hohenheim. The subject of this conference was copper (Cu). Seven German and Austrian scientists who had published and reviewed scientific and regulatory papers on Cu were invited, among them basic researchers, toxicologists, clinical chemists, nutritionists and scientific regulators from the Umweltbundesamt. The participants were presented with 11 questions, which were discussed and answered at the workshop, with the aim of summarising the current state of knowledge on Cu. The explicatory text accompanying the short answers was produced and agreed on after the conference and was backed up by corresponding references. Because of the lack of space, it was not possible to include all references provided by the authors.

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