Effect of copper deficiency on prenatal development and pregnancy outcome

Carl L. Keen, Janet Y. Uriu-Hare, Susan N. Hawk, Margaret A. Jankowski, George P. Daston, Catherine L. Kwik-Uribe, and Robert B. Rucker

ABSTRACT Copper deficiency during embryonic and fetal development can result in numerous gross structural and biochemical abnormalities. Such a deficiency can arise through a variety of mechanisms, including low maternal dietary copper intake, disease-induced or drug-induced changes in maternal and conceptus copper metabolism, or both. These issues are discussed in this article along with the use of in vitro embryo culture models to study the mechanisms underlying copper deficiency–induced teratogenesis. Current data suggest that changes in free radical defense mechanisms, connective tissue metabolism, and energy production can all contribute to the dysmorphogenesis associated with developmental copper deficiency.

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KEY WORDS Copper, development, embryo culture, pregnancy, superoxide dismutase, oxidative damage, fetal development

INTRODUCTION

It is estimated that >50% of human conceptions fail to implant, and, of those implanting, ≈30% fail to reach term. In the United States, ≈3% of infants born have at least one serious congenital malformation, and an average of 10 infants/1000 die before 1 y of life, with about half of these deaths being attributable in part to identifiable birth defects, low birth weight, and prematurity (1). Although advances in developmental and cellular biology have greatly enhanced our understanding of the mechanisms regulating normal development (1, 2), the etiology of most human developmental defects remains to be elucidated.

There is increasing evidence that the origin of a significant number of developmental defects may be because of suboptimal nutrition during embryonic and fetal development (2). This concept is a particularly exciting one because it suggests that the frequency of birth defects, as well as that of other pregnancy complications, may be reduced significantly through appropriate maternal dietary modifications. Consistent with this concept are reports that multivitamin supplements (provided as whole food supplements or as pills) can significantly reduce the frequency of birth defects and maternal complications (3–6). Both the well-established benefit of supplemental iodine for the prevention of cretinism in geographic areas characterized by iodine deficiency (7) and the recent reports on the value of folic acid supplements in reducing the risk of recurrent and occurrent neural tube defects (5, 8–10) serve to underscore the possible benefits that can be obtained through improvements in maternal diet.

As is illustrated in Table 1, deficits of several essential micronutrients have been implicated in the occurrence of select birth defects. In the following review, attention is focused on the teratogenic effects of copper deficiency and the hypothesis that suboptimal copper nutrition during embryonic or fetal development contributes to the occurrence of some human birth defects.

TERATOGENIC CONSEQUENCES OF COPPER DEFICIENCY IN ANIMALS

Evidence for the importance of copper for prenatal development arose from studies of a disease in lambs called enzootic ataxia (also known as swayback). This disorder, which has been reported in numerous countries, including Australia, the United Kingdom, South Africa, and the United States, is characterized by spastic paralysis (especially of the hind limbs), severe uncoordination, and anemia. The brains of affected animals are typically smaller than normal, have collapsed cerebral hemispheres and shallow convolutions, and are hypomyelinated (11–13). In a series of studies, Bennetts et al (14) were able to show that affected animals were typically characterized by low tissue copper concentrations and that the frequency and severity of the disease were markedly reduced when the ewes were given supplemental copper, either before or during early pregnancy (14). Similar neonatal ataxia and brain abnormalities have been reported in newborn copper-deficient goats, swine, guinea pigs, and rats (11–13), further establishing the primary role that copper deficiency has in the development of enzootic ataxia.

The biochemical lesions underlying the developmental brain abnormalities associated with copper deficiency have not been agreed on. One lesion may be a reduction in the activity of the cuproenzyme, cytochrome-c oxidase. Mills and Williams (15) reported that copper deficiency in lambs results in low activity of
TABLE 1
Micronutrients whose deficiencies are postulated as being causative factors in abnormal human prenatal development

<table>
<thead>
<tr>
<th>Vitamin</th>
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<tr>
<td>Vitamin A</td>
<td>Copper</td>
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<tr>
<td>Vitamin B-6</td>
<td>Iodine</td>
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<tr>
<td>Vitamin B-12</td>
<td>Iron</td>
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<tr>
<td>Vitamin D</td>
<td>Magnesium</td>
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<td>Vitamin K</td>
<td>Zinc</td>
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cytochrome-c oxidase in the large motor neurons of the red nucleus of the brain, an area where degeneration is often particularly striking. Although the functional significance of copper deficiency–induced reductions in brain cytochrome-c oxidase activity has not been defined, significant reductions in the activity of this enzyme in the liver and heart have been linked to impaired ATP production (16, 17).

A second mechanism that may contribute to the brain abnormalities associated with copper deficiency is excessive cellular oxidative damage. Brain copper-zinc superoxide dismutase (Cu/Zn SOD) activity has been reported to be low in young, copper-deficient rats (18), and reductions in the activity of this oxidant defense enzyme have been shown to be associated with excessive lipid and protein oxidative damage and cell death (19–24). In contrast with these findings, peroxidation of brain lipids in young, copper-deficient rats has not been reported to be particularly high (18). One explanation for the lack of increased lipid peroxidation in the copper-deficient fetal brain might be that the fatty acid profile has shifted to a less peroxidizable composition. That the amount and type of polyunsaturated fatty acids in adult tissues can change in response to oxidative insults is well established. Presumably, this shift reflects a compensatory response to the oxidative insult (24). However, whether or not a condition of high oxidative pressure can influence the type or amount of polyunsaturated fatty acids in embryonic and fetal tissue has yet to be established. It is important to note that if the fatty acid profile is changed, this in itself may contribute to abnormal development. In addition to peroxidation of lipids, high intracellular concentrations of reactive oxygen species can result in oxidative damage to proteins and nucleic acids; the influence of copper deficiency on the extent and form of protein and DNA oxidative damage in embryonic and fetal brain has not been reported.

In addition to cytochrome-c oxidase and Cu/Zn SOD, the activities of several other cuproenzymes in the brain can be affected by copper deficiency. Prohaska and Bailey (25) reported that in rats, brain peptidylglycine monooxygenase [peptidyl ε-amidating monooxygenase; PAM (EC 1.14.17.3)] is markedly reduced with developmental copper deficiency. Importantly, the brain activity of this enzyme can remain low even after several months of dietary copper repletion. Whether the persistence of low PAM activity in the brain after prenatal copper deficiency is due to the relatively long period of time that it takes to increase brain copper concentrations, or whether it represents a more fundamental epigenetic defect, has not been determined. Regardless of the reason, the persistent reduction in brain PAM activity could have long-term behavioral and metabolic consequences in view of the importance of PAM activity to hormone activation (26, 27). Similar to what has been observed for brain PAM activity, brain cytochrome-c oxidase activity can remain low long after copper repletion (25). In contrast with the above cuproenzymes, dopamine β-monooxygenase activity tends to be higher than normal in the brains of copper-deficient rats than in controls (25).

The extent to which copper deficiency–induced changes in the activities of the above enzymes contribute to the morphologic damage observed in copper-deficient fetal and neonatal brains is not known. However, it is reasonable to postulate that copper deficiency–induced developmental changes in the activities of PAM and cytochrome-c oxidase contribute to the persistent behavioral and functional defects associated with prenatal copper deficiency (26, 27).

Copper and low-molecular-weight copper complexes have angiogenic properties (28). Consequently, it is reasonable to speculate that altered angiogenesis may also contribute to the brain dysmorphology associated with developmental copper deficiency. Given the observations that protein-lysine 6-oxidase (lysyl oxidase) activity can be markedly reduced under conditions of copper deficiency (29, 30), it can also be speculated that compromised vessel integrity, secondary to impaired collagen and elastin cross-linking, could result in abnormal vasculature, hemorrhaging, and blood pooling in the embryonic fetal brain.

It is important to note that whereas the primary cause of enzootic ataxia is clearly embryonic copper deficiency, fetal copper deficiency, or both, mothers may often appear to be quite healthy, showing no obvious signs of copper deficiency (14). Similarly, pregnant copper-deficient rats and mice often show no obvious signs of deficiency, even when their litters are severely affected (11, 12).

Another interesting observation, drawn from work on enzootic ataxia, is that the occurrence of the disorder can be dramatically influenced by the genetic background of the ewes. For example, when Welsh and Blackface sheep were grazed on the same low-copper pasture, there were no cases of the disease in the offspring of the Welsh ewes, whereas 40% of the offspring of the Blackface ewes were affected. In contrast, when Cheviot ewes were grazed on the same pasture, their offspring had an incidence of enzootic ataxia of 11% (31). These differences in the occurrence of this developmental defect are thought to be due to breed (maternal) differences in copper absorption, retention, or both (31). The differences by strain in copper metabolism observed in these sheep are thought to involve multiple genes. Given that significant strain differences in response to copper deficiency have also been reported for rats, mice, and chickens (11, 32–34), it is reasonable to suggest that similar variations in the response to copper deficiency during pregnancy probably occur in the human population at both the adult and conceptus level.

In addition to brain defects, copper-deficient fetuses and neonates are typically characterized by severe connective tissue abnormalities. Cardiac hemorrhages are a frequent finding in copper-deficient sheep, rats, guinea pigs, and mice (11, 29, 30). The walls of the internal and common carotid arteries in deficient fetuses tend to have an endothelium that is normal in appearance, but with sparse, poorly developed elastin. Cerebral arteries are also often characterized by a low elastin content. Furthermore, the elastin that is present does not have the concise fibril arrangements seen in copper-replete animals. This reduction in elastin content and cross-linking integrity is thought to be due primarily to a decrease in the activity of lysyl oxidase, a copper-containing enzyme that catalyzes the oxidation of certain peptide lysine and hydroxylysine residues to peptide aldehydes, initiating the cross-
linking mechanisms required for connective tissue stability (29, 30). In addition to the reduction in cross-linking, copper deficiency can be associated with an accelerated proteolysis of collagen and elastin due to nongenetic proteases that migrate from the blood compartment into vascular tissue (35, 36).

Skeletal defects also often occur as a result of prenatal copper deficiency. Lambs with enzootic ataxia may have poorly developed, light, brittle bones, and frequent fractures. Bone abnormalities have also been found in copper-deficient calves and birds. In dogs and swine, it was observed that offspring born to females fed copper-deficient diets had deformed leg bones (11). The lesions appeared to be associated with an impairment of osteogenesis, with the resultant thinning of the cortex and trabeculae of the long bones. Copper-deficient chicks had severe hypoplasia of the long bones. With copper deficiency, bone amine oxidase and cytochrome-c oxidase activities are low and there is a high ratio of soluble to insoluble collagen. The increased fragility of copper-deficient bones is thought to result from the low number of cross-links present in the collagen’s matrix (11, 30).

Lung abnormalities are also a frequent consequence of prenatal and early postnatal copper deficiency. Lungs from neonatal rabbits born to dams fed copper-deficient diets are characterized by low concentrations of copper, low lysyl oxidase activity, and high proportions of poorly cross-linked elastin and collagen. The lungs are also characterized by low concentrations of surfactant phospholipids (37). Similar results have been reported for copper-deficient rat pups born to dams fed either low-copper diets or diets containing the copper-chelating drugs D-penicillamine (DPA) or triethylenetetramine (TETA) (38, 39).

HUMAN COPPER DEFICIENCY AND TERATOGENESIS

It is estimated that the average intake of copper by women of childbearing age is lower than the current estimated safe and adequate daily intake for adults, which is 1.5–3.0 mg Cu/d (40). Whereas overt copper deficiency in adult humans is relatively rare, signs of moderate copper deficiency are thought to be more common (41–47). Copper deficiency in human infants has been detected under a variety of conditions (44–47); however, the extent to which copper deficiency influences human prenatal development is a subject of considerable debate. Morton et al (48) reported a significant correlation between low copper in drinking water and the occurrence of neural tube defects in South Wales, with the implication being that a primary deficiency of copper could result in birth defects in humans. Although this is an intriguing observation, more data in other settings are clearly needed before generalizations can be made. However, it is important to note that consistent with the above report, Buamah et al (49) observed that low serum copper concentrations in pregnant women during midgestation were associated with an increased risk for anencephaly.

Although the development of a condition of copper deficiency during pregnancy as a consequence simply of an inadequate dietary intake of the element (primary deficiency) may be rare, secondary or conditioned copper deficiencies can arise by several means (Table 2). For example, as described above, genetic factors can influence an individual’s copper requirement, nutritional interactions can produce conditioned copper deficiencies, drugs or other chemicals can precipitate the development of deficiencies, and several disease states can alter copper metabolism and in doing so create the potential for a deficiency state. Examples of each of the above are presented below.

**TABLE 2** Causative factors in developmental copper deficiency

| Primary deficiency—ineffective maternal dietary copper intake |
|-------------|----------------|
| Secondary deficiency | Gene defects |
| Mutant genes (Menkes syndrome; occipital horn syndrome) | Strain differences (breed variation in susceptibility to enzootic ataxia) |

<table>
<thead>
<tr>
<th>Nutritional interactions</th>
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<tbody>
<tr>
<td>Copper-trace metal interactions (Zn, Cd, and Ag)</td>
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<tr>
<td>Copper interactions with other nutrients (copper-fructose interactions)</td>
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</tbody>
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<table>
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<tr>
<th>Drugs or other chemicals</th>
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<tbody>
<tr>
<td>Direct copper chelation (DPA, TEMA)</td>
</tr>
<tr>
<td>Altered copper metabolism (alcohol, diuretics, laxatives)</td>
</tr>
<tr>
<td>Disease-associated changes in copper metabolism (diabetes, hypertension)</td>
</tr>
</tbody>
</table>

1 DPA, D-penicillamine; TEMA, triethylenetetramine.

**GENETIC DEFECTS AND COPPER**

As was discussed above, there can be significant variations among animal strains (breeds) in the response to copper deficiency (31). Whereas breed variation, particularly in sheep, is thought to be due to multiple genes, defects centered around a single gene involving copper metabolism have also been reported. Illustrative of these are the mottled (Mo) mouse mutants, which are characterized by defects in cellular copper transport (50, 51). More than 10 alleles at the mottled locus have been described, the phenotypic expression of which ranges in severity from hypopigmentation to death in utero. The blotchy (Mo<sup>bl</sup>) mutant is particularly noteworthy because it is characterized by severe connective tissue defects and neurologic abnormalities, which are similar to those observed with severe maternal copper deficiency (51).

Genetic defects resulting in conditions of copper deficiency also occur in humans. Two severe genetic disorders in humans that influence prenatal development are Menkes syndrome and occipital horn syndrome, both of which are thought to be due in part to defects in a copper-transporting ATPase gene (32, 51, 52). Infants with Menkes syndrome are characterized by progressive degeneration of the brain and spinal cord, hypothermia, connective tissue abnormalities, and failure to thrive. Although this disease has been recognized to be a disorder of copper metabolism for >20 y, the prognosis for infants with this disorder is still poor, with death typically occurring by 3 y of age (32). The developmental abnormalities associated with Menkes syndrome are thought to be due to low activities of numerous copper enzymes during embryonic and fetal development; however, as is discussed below, the exact biochemical lesions underlying the observed abnormalities are still poorly defined. In contrast with patients with Menkes syndrome, individuals with occipital horn syndrome have been reported to be phenotypically normal at birth, but detailed biochemical studies of these infants at birth have not been reported (32).

A third genetic defect in humans that mimics, in part, copper deficiency is aceruloplasminemia. This disorder is characterized by a mutation in the ceruloplasmin gene, which results in very...
low, or no, ceruloplasmin production (53, 54). The effect of this defect on embryonic and fetal development has not been defined, although it is important to note that individuals with this defect do conceive.

**Nutritional interventions and copper**

With respect to dietary interactions, consumption of high concentrations of zinc in the diet can induce a secondary copper deficiency as a consequence of the similar physiochemical properties of these two elements (55, 56). The interaction between zinc and copper is of practical concern because it can occur with relatively low amounts of zinc supplementation (55, 56). To date, a zinc-induced copper deficiency has yet to be reported to negatively affect a human pregnancy. Despite this, the observation that supplemental zinc–induced copper deficiencies are relatively easy to produce in adult humans (55, 56), coupled with the observation that in experimental animals maternal zinc supplementation can induce fetal copper deficiency (57), it is reasonable to argue that caution should be taken when pregnant women are given zinc supplements. Consistent with this, the Institute of Medicine has recommended that copper supplements should be provided when zinc supplements are given during pregnancy (58). Unfortunately, this recommendation (along with the recommendation that zinc supplements should be given when iron supplements are prescribed) has not been widely publicized or enforced.

Copper deficiency can also be induced by consumption of high concentrations of molybdenum, which, along with sulfur, can form a complex with copper that limits its absorption. Howell et al (59) have shown that a condition of severe copper deficiency can be induced in pregnant guinea pigs through the use of molybdenum-supplemented diets. In rats, fetal copper deficiency can also be induced by feeding the mother high concentrations of cadmium (60). Recently, Shavlovski et al (61) reported that in rats, embryonic copper deficiency can also occur as a consequence of maternal silver toxicity. The mechanism underlying this effect of silver has not been defined, although the authors suggested that it involves perturbations in normal ceruloplasmin production in the maternal liver. This is somewhat enigmatic in view of evidence evolving from studies on aceruloplasminemia in humans. This suggested mechanism, if shown to be correct, is exciting because it would argue for the concept that ceruloplasmin is a significant vehicle for the delivery of copper to the conceptus.

An additional dietary component that may influence the rate at which a copper-deficiency state occurs is the mixture of carbohydrates in the diet. FIELDS et al, in a series of studies (62, 63), reported that the presence of fructose in a diet will significantly accelerate the development of copper deficiency when low-copper diets are fed. Fields et al (62) reported that the teratogenicity of copper-deficient diets is greatly increased when a diet contains fructose in place of starch. Although the above work has been done primarily with rodent models, the extent to which fructose-copper interactions influence human pregnancy outcomes needs to be defined clearly.

**Drug interactions and copper**

Conditions of severe copper deficiency can be rapidly induced through the use of several chelating drugs, including disulfiram (antebuse and diethyl dithiocarbamate), DPA, TETA, and meso-2,3-dimercaptosuccinic acid (DMSA) (64–68). All of these drugs have been shown to be teratogenic in animal models, with the teratogenic expression of each being consistent with that occurring with dietary copper deficiency. The teratogenic expression of TETA and DPA in animal models has been shown to be modulated by maternal dietary copper intake (69, 70). However, it is important to note that these chelating agents typically influence the metabolism of other divalent metals (eg, zinc) or act as metabolic inhibitors; thus, their teratogenicity should not be ascribed to their effects on copper alone.

Rosa (71) reviewed a series of cases of infants born to women who received DPA during pregnancy. Abnormalities observed in these infants included lax skin, hyperflexibility of the joints, fragility of the veins, and numerous soft tissue abnormalities. It was suggested that the above malformations were in part due to a drug-induced copper deficiency during embryonic or fetal development; similar abnormalities were produced with DPA in rodent models (66), and the teratogenicity of the drug is modulated by maternal dietary copper intake (70).

The influence of other copper-chelating drugs—such as disulfiram, captopril, TETA, and DMSA—on human prenatal development remains to be determined. However, given the significant teratogenic effects that can be associated with such drugs in experimental animals, it is reasonable to suggest that these drugs can pose a significant risk to the conceptus if maternal dietary copper intake is low.

**Disease states and copper**

Finally, there are several disease states that can be characterized by alterations in copper metabolism, including chronic diarrhea, diabetes, alcoholism, and hypertension (72, 73). It has been speculated that the teratogenesis associated with maternal diabetes and maternal alcoholism may be due in part to disease-induced deficiencies of copper in the embryo or fetus (74, 75). Consistent with the above, Speich et al (76) reported low erythrocyte copper concentrations in infants of diabetic mothers. In addition to the above diseases, maternal hypocupremia is often noted in cases of spontaneous abortion and postmaturity and premature rupture of the fetal and placental membranes (77, 78).

**MECHANISMS OF TERATOGENICITY OF COPPER DEFICIENCY**

It is evident from the foregoing studies that severe maternal copper deficiency can result in embryonic and fetal abnormalities; however, the mechanisms underlying these defects are not well understood. Some of the basic mechanisms that have been postulated to contribute to copper deficiency–associated teratogenicity are shown in Table 3. A fundamental question is whether the effects of copper deficiency on the embryo or fetus are due directly to a deficiency of copper in embryonic or fetal cells, or whether they occur in part through indirect effects of copper deficiency on the metabolism of the mother (eg, as a consequence of maternal anemia, tissue iron accrual, or accelerated turnover or production of key pregnancy hormones or growth factors). Although the observation that consumption of a copper-deficient diet results in low copper concentrations in maternal plasma, as well as in embryonic and fetal tissues, suggests a direct effect of copper deficiency on the conceptus, it does not prove a causal relation between low fetal copper concentrations and malformations.

One approach to the study of the direct effects of copper deficiency on the embryo is through the use of embryo culture sys-
either copper-adequate (either control or copper-deficient dams were cultured for 48 h in serum obtained from control or nutrient-deficient (or toxicant-treated) rats. With this system, our group (85) reported initially that when rat embryos are cultured in vitro for up to 72 h, by which time they have reached the blastocyst stage. Although preimplantation embryo systems have been used to study the effects of zinc deficiency on early development (79–81), this model has not been widely exploited for the study of copper deficiency–associated teratogenicity. However, in a preliminary report by Menino et al (82), it was observed that preimplanted mouse embryos collected from dams that had been fed copper-deficient diets for 2 mo before mating showed an impaired ability for hatching (blastocyst escape from the zona pellucida) when they were cultured in vitro. This is an exciting report that needs to be pursued. Note that the preimplantation embryo model has also proved to be useful in the study of the developmental toxicity of excess copper (83, 84).

The second model that is commonly used is the postimplantation embryo culture technique. With this system, embryos (rat or mouse) are removed from control (or nutrient deprived) dams during early development (typically from gestation day 8–10); they are then cultured for up to 48 h in medium obtained from control or nutrient-deficient (or toxicant-treated) rats. With this system, our group (85) reported initially that when rat embryos are cultured in copper-deficient serum for 48 h they are smaller than normal and are characterized by narrow optic sulci and an ill-defined otic placode. We verified recently and extended the above work (86). In our recent study, embryos (gestation days 9 and 10) obtained from either control or copper-deficient dams were cultured for 48 h in either copper-adequate (20 μmol/L) or copper-deficient (2.5 μmol/L) serum. The control embryos cultured in control serum were morphologically normal. In contrast, copper-deficient embryos cultured in copper-deficient serum displayed numerous abnormalities, including swollen hindbrains, blisters, blood pooling, and distention of the large vessels, particularly the anterior cardinal veins. A typical control embryo and a copper-deficient embryo showing a swollen hindbrain are shown in Figure 1, A and B. Control embryos cultured in copper-deficient serum and copper-deficient embryos cultured in control serum showed intermediate results. It is important to note that developmental abnormalities similar to those observed for copper-deficient embryos cultured in copper-deficient serum were observed in embryos obtained in vivo from dams fed copper-deficient diets on gestation day 11 (87). For example, as shown in Figure 1, the gross appearance of copper-deficient embryos obtained from the in vitro model is strikingly similar to that of embryos obtained from copper-deficient dams on gestation day 11 (panel D). It can be observed that the in vitro and in vivo control embryos are also similar morphologically (panels A and C).

The above results support the concept that embryo culture systems, particularly the postimplantation system, can be used to study the mechanisms underlying copper deficiency–induced abnormal development. Toward this end, we recently measured SOD activity in control and copper-deficient embryos cultured in control and copper-deficient serum (86). The copper-deficient group was characterized by very low SOD activities, compared with control embryos (≈40% lower). When reactive oxygen scavengers (SOD or glutathione peroxidase) were added to the culture media, embryonic development was improved, suggesting that the copper deficiency–induced reduction in embryonic SOD activity was functionally significant. It is important to note that despite the apparent elevation of reactive oxygen species in the copper-deficient embryos, these embryos are not characterized by evidence of excessive cell death (87). Currently, we are analyzing copper-deficient and control embryos for indexes of oxidative damage such as DNA-protein cross-links, protein oxidative damage, and low glutathione concentrations (86).

**TABLE 3**

Mechanisms that may contribute to copper deficiency–associated teratogenicity

<table>
<thead>
<tr>
<th>Indirect</th>
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<tr>
<td>Copper deficiency–induced changes in maternal metabolism</td>
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<table>
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<tr>
<th>Direct</th>
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<tr>
<td>Altered extracellular matrix (eg, low lysyl oxidase activity)</td>
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<tr>
<td>Excessive oxidative damage (eg, low superoxide dismutase activity)</td>
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<tr>
<td>Impaired energy production (eg, low cytochrome-c oxidase activity)</td>
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<tr>
<td>Altered angiogenesis</td>
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<td>Low activities of other copper enzymes</td>
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(see panel B) is strikingly similar to that of embryos obtained from copper-deficient dams on gestation day 11 (panel D). It can be observed that the in vitro and in vivo control embryos are also similar morphologically (panels A and C).

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That a compromised oxidant defense system could be a factor in copper deficiency–associated teratogenicity is consistent with recent suggestions that imbalances in reactive oxygen species (either as a consequence of their excessive production or their impaired clearance) may underlie the teratogenic actions of numerous compounds (88). In this regard, it is worth noting that the teratogenicity of diabetes is reduced in mice that are transgenic for the overexpression of Cu/Zn SOD (89). The above observation is particularly interesting in light of reports that maternal diabetes can be associated with low fetal copper concentrations (74, 90), although it is important to note that this is not a universal finding (91). Low fetal SOD activity secondary to low fetal copper concentrations has also been postulated to contribute to the teratogenicity of alcohol (75).

Although it is tempting to suggest that an increase in cellular oxidative damage secondary to a compromised oxidant defense system is a primary biochemical lesion underlying the teratogenicity of copper deficiency, the activities of several other key cuproenzymes are undoubtedly reduced. Indeed, the activities of most cuproenzymes have been shown to be altered by copper deficiency in growing animals (Table 4), although the ontogeny of these enzymes is not yet known, nor is the time when their activities can first be affected by copper deficiency. We obtained preliminary data, similar to that for SOD activity, that suggest that cytochrome-c oxidase activity is markedly reduced in embryos grown in copper-deficient serum (≈50% lower in deficient embryos than in controls), suggesting that these embryos may be characterized by low ATP production. The observation that the treatment of sea urchin embryos with β-aminopropionitrile, an irreversible inhibitor of lysyl oxidase, results in developmental arrest at the mesenchyme blastula stage (92), argues that some of the early developmental defects observed with copper deficiency can also be attributed to copper deficiency–associated reductions in lysyl oxidase activity.

Given the multiplicity of roles that copper plays during embryonic and fetal development, the teratogenicity of copper deficiency likely involves an impairment of more than one pathway or process. The structural and biochemical defects (both short term and epigenetic) associated with developmental copper deficiency must arise from several biochemical lesions, the significance of each being related to their temporal expression and severity. It is important to note that, in addition to the determination of the effect of copper deficiency on the activities of cuproenzymes in embryonic and fetal tissue, the activities of several noncuproenzymes that are known to be influenced by copper deficiency [e.g., catalase, glutathione peroxidase, glutathione transferase, and 3-hydroxy-3-methylglutaryl coenzyme A reductase (21, 93, 94)] should also be assessed in studies aimed at defining the biochemical lesions that underlie the teratogenic effects of copper deficiency.

**TERATOGENESIS OF EXCESS COPPER**

It is well known that small amounts of copper from intrauterine devices can prevent embryogenesis by blocking implantation and blastocyst development. Fern and Hanlon (95) and DiCarlo (96) reported that copper injected in hamsters on day 8 of pregnancy is teratogenic, resulting in thoracic and ventral hernias, microphthalmia, cleft lip, pulmonary hypoplasia, and ectopic cordis. Similarly, a high incidence of fetal loss was reported for rats given copper injections from days 7 through 10 of pregnancy (97). However, the teratogenicity of excess dietary copper in mammals has yet to be established (13, 98–100). Similarly, copper released from intrauterine devices has not been reported to be teratogenic once implantation has occurred (13, 98–100). There are no consistent reports of abnormalities in the offspring of mothers of untreated Wilson disease (a disease that is characterized by high tissue copper concentrations), although pregnant women with untreated Wilson disease often have spontaneous abortions. In addition, fetal tissue copper concentrations can be elevated in Wilson disease patients (101).

**CONCLUSIONS**

Pregnancy is associated with extraordinary metabolic demands on both the mother and developing fetus. In this review, evidence was presented that adequate maternal copper nutriture is essential for normal embryogenesis. Studies in laboratory animals as well as in domestic animals under field conditions have shown that maternal copper deficiency produces effects ranging from intrauterine growth retardation and teratogenesis to embryonic or fetal death. Persistent postnatal complications of prenatal copper deficiency can also occur.

Although a critical role for copper in human development is well established from studies on Menkes syndrome and occipital horn syndrome, the extent to which maternal dietary copper deficiency influences human pregnancy outcome has not been studied in depth. However, there is evidence that disease- and drug-induced alterations in maternal copper metabolism may result in pregnancy complications in these high-risk groups.

Future studies need to be directed toward 1) a better understanding of the mechanisms underlying the teratogenicity of copper deficiency, 2) an improvement in techniques for the determination of maternal and infant copper status, 3) the determination of the effect of modest copper supplementation during pregnancy on the risk of pregnancy complications and fetal outcome in large population groups, and 4) the development of optimal therapies for the treatment of infants who are copper deprived prenatally.

**REFERENCES**


**TABLE 4**

Cuproenzymes whose activities change after copper deficiency

<table>
<thead>
<tr>
<th>Enzyme Name</th>
<th>Description</th>
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<tbody>
<tr>
<td>Amine oxidase</td>
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<tr>
<td>Cerebroplasmin</td>
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<td>Cytochrome-c oxidase</td>
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<tr>
<td>Dopa oxidase</td>
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<tr>
<td>Dopamine-β-monooxygenase</td>
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<tr>
<td>Extracellular superoxide dismutase</td>
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<td>Lysyl oxidase</td>
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<td>Peptidylglycine-α-amidating monooxygenase</td>
<td></td>
</tr>
<tr>
<td>Cu/Zn-superoxide dismutase</td>
<td></td>
</tr>
<tr>
<td>Tyrosinase</td>
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COPPER DEFICIENCY DURING PREGNANCY


