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Development of Glutathione Peroxidase Activity During Dietary and Genetic Copper Deficiency

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Abstract

Copper deficiency was produced in developing rodents to study a possible interaction between copper and the selenoenzyme, glutathione peroxidase (GSH-Px). Dietary copper deficiency was investigated in Sprague-Dawley rats and in three mouse strains (C57BL, C3H/HeJ, C58); genetic copper deficiency was studied in two of the mouse strains, C57BL and C3H/HeJ, using brindled mice. In certain cases it appeared that copper deficiency was associated with depressed liver GSH-Px activity; values from copper-deficient livers were 40–70% of control values. However, the decrease in liver GSH-Px in both rats and mice was only observed when body weight was also depressed and did not necessarily correlate with copper deficiency signs, such as lower serum ceruloplasmin or liver cytochrome oxidase activities. In weanling rats, serum GSH-Px activity was normal despite a 60% reduction in liver activity. Mouse liver GSH-Px activity rose fourfold during the first 3 weeks of life to 75% of the adult level. Rat liver GSH-Px also increased during the suckling period. When perinatal copper deficiency, nutritional or genetic, was severe enough to retard growth, liver GSH-Px activity was depressed. Unlike rats, adult murine liver GSH-Px was equivalent in males and females.

Index Entries: Brindled mice; copper-deficient mice; copper-deficient rats; copper deficiency and glutathione peroxidase; mouse liver glutathione peroxidase and age; copper-deficiency and liver enzymes; rats, copper deficient; glutathione peroxidase, and copper deficiency; liver enzymes, and copper deficiency; enzymes, liver, and copper deficiency.

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Introduction

Copper has many biochemical functions in eukaryotic organisms that are expressed by a variety of cuproenzymes in many cell types and in several subcellular compartments. One such cuproenzyme is superoxide dismutase (EC 1.15.1.1) (SOD), which contains 2 mol each of copper and zinc in the active dimer and is found in the cytoplasm and mitochondrial intermembrane space (*1*). SOD catalyzes the disproportionation of the superoxide radical (O^-_2) to hydrogen peroxide and molecular oxygen and serves to protect aerobic cells from reactions dependent on aberrant levels of O^-_2, such as the initiation of lipid peroxidation (*2*).

Dietary copper deficiency in rats results in reductions in SOD activity in brain (*3–5*), liver (*6–10*), heart (*6–8*), erythrocytes (*6–8, 11–13*), and other tissues (*6*). The consequences of this SOD reduction are less clear. No evidence for enhancement of lipid peroxidation was obtained in brain studies (*4*). Erythrocyte hemolysis is actually lower in copper-deficient (−Cu) preparations (*8, 12, 13*), yet erythrocyte membranes from −Cu rats have an increased spontaneous and induced peroxidation of lipids as measured by malondialdehyde production (*13*). Liver mitochondria from −Cu rats are characterized by increased lipid peroxidation (*7, 9, 14*), but the mechanisms may involve more than low SOD levels. Isolated −Cu rat liver mitochondria have decreased concentrations of total phospholipids, which may alter membrane integrity, and contain more polyunsaturated fatty acids, substrates for lipid peroxidation (*15*). Furthermore, the −Cu mitochondria have decreased levels of SH groups (*16*). It is not known if any of these factors are responsible for the increased lipid peroxidation measured in vitro.

Recently another factor has been suggested to modify the −Cu liver toward enhanced lipid peroxidation. Balevska et al. (*9*) fed young rats a −Cu diet based on powdered milk, and found that the activity of the selenoenzyme, glutathione peroxidase (EC 1.11.1.9) (GSH-Px), was greatly depressed in liver compared to rats fed a "standard laboratory diet" containing 15 ppm copper. Jenkinson et al. (*10*) found similar results using a −Cu diet based on evaporated milk. In contrast, others (*8, 18*) using a purified diet adequate in all nutrients except copper have found no difference in liver GSH-Px activities in −Cu rats. Preliminary studies in suckling mice indicated that the decrease in liver GSH-Px may not correlate with the degree of copper deficiency, since brindled mice, which are copper-deficient because of a mutation of the *X*-chromosome (*17*), have low liver GSH-Px activity but normal activity of the cuproenzyme, cytochrome oxidase (EC 1.9.3.1) (*18*). In contrast, mice made deficient in copper by dietary means had normal liver GSH-Px, but lower cytochrome oxidase activities (*18*). The present experiments were conducted to extend these observations in mice and investigate the relationship regarding copper deficiency and GSH-Px activity in young rats.

Materials and Methods

Animal Care and Diets

Genetic copper deficiency was studied in brindled mice, which have a mutation at the mottled locus of the *X*-chromosome (*17*). Hemizygous brindled males

($Mo^{br/y}$) were obtained from matings of heterozygous females ($Mo^{br/+}$) with normal males ($Mo^{+/y}$). Two strains of mice were used, C3H/HeJ (Jackson Laboratories, Bar Harbor, ME) and C57BL (kindly provided by Douglas Grahn, Argonne National Laboratory). These genetic breeding units were given tap water to drink and fed a nonpurified diet (mouse chow, Ralston Purina) containing 12–14 ppm of copper by analysis. Brindled males die when about 2 weeks old and thus were studied prior to this age, usually when 11–12 d old.

Dietary copper deficiency was produced by feeding a purified diet low in copper, modified AIN-76A (Teklad Laboratories, Madison, WI), formulated to omit cupric carbonate from the salt mix. When supplemented with copper, this diet provides adequate nutrition for growing mice and rats (*19*). The diet is based on sucrose (50%), casein (20%), cornstarch (15%), corn oil (5%), cellulose (5%), mineral mix (3.5%), and vitamin mix (1%). The copper-deficient diet averages 0.5 ppm copper and 45 ppm iron by analysis. The diet was fed, beginning with the putative gestational period, to normal females ($Mo^{+/+}$) and males ($Mo^{+/y}$) of both mouse strains in which the mutants were studied. A third strain was also studied, C58, for comparison. Half the breeding units and subsequent dams were given supplemental copper in their drinking water as cupric sulfate (20 ppm copper), and their offspring served as controls (+Cu). The other dams received deionized water to drink and their offspring are referred to as copper-deficient (−Cu). Similar studies have been conducted previously (*20, 21*). An age study was conducted in offspring of C57BL mice fed the nonpurified diet and tap water.

Dietary copper deficiency was also produced in offspring of albino rats (Sprague Dawley, Madison, WI) by feeding sperm-positive females the purified diet and deionized water (−Cu) during gestation and lactation. Copper-supplemented (+Cu) dams were included as in the mouse studies. Ten dams, five +Cu and five −Cu) and their subsequent litters were studied. Some rat offspring were transferred to stainless steel cages when 3 weeks old and were continued on their respective treatments. Three additional experiments were conducted in which 20 male weanling rats were divided equally for the −Cu and +Cu treatments for 2 weeks. They were housed individually in stainless steel cages and samples of four rats of each treatment were taken for each of the three experiments. More than 50 rats and 200 mice were sampled for this study.

Enzyme Assays

Most biochemicals for enzymatic studies were obtained commercially (Sigma Chemical Co., St. Louis, MO). Two cuproproteins were estimated by measuring their catalytic activities. Serum ceruloplasmin (EC 1.16.3.1) was measured using *o*-dianisidine as substrate, as described previously (20). Mouse serum does not yield reliable data when *p*-phenylenediamine is used as substrate because of heat-stable copper-independent oxidase activity. Liver cytochrome oxidase (EC 1.9.3.1) was determined on fresh homogenates by following loss of ferrocytochrome *c* at 550 nm (Beckman DU-8 spectrophotometer), as described previously (*3*) for brain tissue.

Two noncuproenzymes were estimated in liver. Fumarase (EC 4.2.1.2) activity was measured spectrophotometrically by following fumarate formation at 240 nm

by the method of Racker, as previously described (*4*). Glutathione peroxidase (EC 1.11.1.9) GSH-Px activity was determined spectrophotometrically at 340 nm by modifying a coupled enzyme procedure originally developed by Paglia and Valentine. The assay contained 100 m*M* HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.5), 0.1 m*M* EDTA, 1 m*M* GSH, 0.11 m*M* NADPH, 4 μg of yeast glutathione reductase, and a sample of a detergent-treated supernate (typically for liver, 0.002 mL of a 5% solution) that was preincubated at 37°C for 5 min before the reaction was brought to 1.0 mL with 0.02 mL of 12.5 m*M* *t*-butylhydroperoxide dissolved in ethanol (0.25 m*M* final) (K & K Laboratories, Plainview NY). Under these conditions, maximum sensitivity for GSH-Px is maintained and the error caused by the glutathione peroxidase activity of GSH transferases (*22*) is minimal.

Livers were homogenized in nine volumes of 0.32*M* sucrose containing 0.1 m*M* EDTA (pH 7.0), and aliquots were diluted with detergents to measure total activity—Tween-80 for cytochrome oxidase measurement (*3*) or Triton X-100 for fumarase (*4*) and GSH-Px (*23*) measurement. Most determinations of liver GSH-Px activity were done on liver samples that were homogenized in 19 volumes of a solution (pH 7.5) containing 100 m*M* KCl, 20 m*M* Tris, 0.1 m*M* EDTA, and 0.5% Triton X-100. The homogenate was centrifuged, 8000*g* for 10 min, and an aliquot of the supernate was assayed for GSH-Px activity. Liver protein was analyzed on total homogenates or supernates by a modified Lowry technique (*24*) using bovine albumin as a reference. Activities of all four enzymes were expressed in units (μmol/min) per mg liver protein or liter of serum.

Statistical Analysis

Population means were compared using the *F*-variance ratio and Student's *t*-test by means of a computer program (SPSS). Statistical significance was tested at $\alpha = 0.01$.

Results

Recent studies (*20, 21*) in our laboratory indicated that by feeding dams a purified diet low in copper, dietary copper deficiency could be produced in young mice. The response of this treatment was strain-dependent (Table 1). In two of the strains, C57BL and C58, the copper-deficient pups (−Cu) were smaller than the copper-supplemented (+Cu) pups. In the other strain, C3H/HeJ, the −Cu and the +Cu pups were equivalent in body weight.

Several liver enzymes were studied in these mice. The −Cu mice of all three strains exhibited signs of copper deficiency, since all −Cu offspring had depressed liver cytochrome oxidase activities compared to the +Cu controls (Table 1). The smaller −Cu mice, C58 and C57BL, had significant decreases (about 30%) in liver GSH-Px activity, whereas the −Cu C3H/HeJ offspring had activities equivalent to their controls (+Cu).

Similar studies were made in offspring of mutant dams carrying the brindled gene (Table 1). Normal male offspring ($Mo^{+/y}$) from the affected dams were simi-

TABLE 1

Effect of Dietary and Genetic Copper Deficiency on Body Weight and Liver Enzymes in Mice[a]

Strain/treatment		Body wt, g	Glutathione peroxidase, units/mg	Cytochrome oxidase, units/mg	Fumarase,[b] units/mg
C57BL	+Cu (*14*)	5.1 ± 0.8	1.40 ± 0.27	0.321 ± 0.047	—
	−Cu (*14*)	3.7 ± 0.8*	0.939 ± 0.222*	0.127 ± 0.013*	—
	$Mo^{+/y}$ (*20*)	5.8 ± 1.0	1.26 ± 0.433	0.490 ± 0.146	0.617 ± 0.036
	$Mo^{br/y}$ (*19*)	3.8 ± 0.6*	0.702 ± 0.243*	0.479 ± 0.110	0.662 ± 0.034
C3H/HeJ	+Cu (*5*)	6.4 ± 1.2	1.11 ± 0.25	0.560 ± 0.151	—
	−Cu (*7*)	6.5 ± 0.8	1.00 ± 0.12	0.125 ± 0.034*	0.623 ± 0.016
	$Mo^{+/y}$ (*6*)	6.7 ± 0.9	1.43 ± 0.21	0.626 ± 0.084	0.678 ± 0.160
	$Mo^{br/y}$ (*3*)	3.0 ± 0.1*	0.67 ± 0.12*	0.502 ± 0.082	0.622 ± 0.128
C58	+Cu (*4*)	5.9 ± 0.1	0.972 ± 0.089	0.481 ± 0.024	—
	−Cu (*2*)	2.7,4.0	0.540,0.810	0.313,0.288	—

[a]Copper deficiency was studied in three mouse strains in 11–12-d-old male mice. Dietary copper deficiency was produced by feeding normal mouse dams a diet deficient in Cu during gestation and lactation. Their offspring (−Cu) were compared to control offspring (+Cu) from dams fed the same diet but they were given supplemental copper in the drinking water, 20 ppm. Genetic copper deficiency was studied in male mice derived from dams heterozygous for the brindled allele at the mottled locus of the *X*-chromosome. Affected brindled males ($Mo^{br/y}$) were compared to their normal littermate brothers ($Mo^{+/y}$). Heterozygous dams were fed a nonpurified diet containing 12 ppm copper and were given tap water to drink. Values are means ± SD for the number of mice indicated, which were random samples from 2–5 litters of mice; an * indicates that means between respective groups (+Cu vs −Cu, $Mo^{+/y}$ vs $Mo^{br/y}$) were different when compared by Student's *t*-test, $P < 0.01$.

[b]Fumarase was determined on a smaller sample set (5–6 livers) and no statistical mean differences existed

lar in size to the +Cu mice in both strains, C57BL and C3H/HeJ, that were studied. The brindled male offspring ($Mo^{br/y}$) were smaller than their littermate brothers ($Mo^{+/y}$) in both strains and equivalent in body weight to the −Cu mice of the C57BL strain (Table 1).

Analysis of liver enzymes was also conducted for the brindled mice and their controls. The activity of liver cytochrome oxidase was not statistically lower in $Mo^{br/y}$ mice compared to $Mo^{+/y}$ (Table 1); however, the activity of another cuproenzyme, superoxide dismutase, was decreased by 20% in liver of $Mo^{br/y}$ C57BL mice (unpublished). This decrease was much less than that observed in C57BL −Cu mice (53% decrease) compared to +Cu mice (unpublished). Fumarase activity was not altered in $Mo^{br/y}$ mouse liver, but in both C57BL and C3H/HeJ mice, the $Mo^{br/y}$ liver GSH-Px activities were significantly decreased 44 and 53% respectively, compared to $Mo^{+/y}$ mice (Table 1).

The decreases in liver GSH-Px activity in −Cu and $Mo^{br/y}$ mice correlated better with reductions in growth (body weight) than with copper deficiency status (Table 1). Preliminary studies had indicated that the level of mouse liver GSH-Px activity was dependent on age (*18*). Therefore, a more thorough study was conducted in C57BL mice to determine the effect of age on liver GSH-Px activity (Fig. 1). Suckling mice were nursed by normal dams fed the nonpurified diet, and litter size was fixed at eight pups per dam. There was a fourfold rise in liver GSH-Px activity between birth and weaning (age 3 weeks) with the largest increment occuring during the second 2 postnatal weeks (Fig. 1A). The activity of liver GSH-Px at 20 d of age was about 75% of the adult value. GSH-Px activity in adult male and female liver was equivalent, 2.38 ± 0.29 (N = 8) units/mg protein. The growth pattern of the C57BL offspring was strikingly similar to the liver GSH-Px profile (Fig. 1B). The mean body weight at 20 d of age was only 25% of the adult weight of 34.6 g. The variability in liver GSH-Px activity in 11–12 d old mice (Table 1) was caused, in part, by the rapidly changing activity during perinatal development (Fig. 1).

In previous studies on GSH-Px, copper deficiency was produced in rats by feeding weanling animals a −Cu diet for 4–8 weeks (*7–10*, *18*). The current studies were conducted in younger rats during the suckling period up to 5 weeks of age. Copper deficiency in the dams was reflected in their offspring in modest growth impairment at 12 d of age and more pronounced impairment at 20 d of age (Table 2). The −Cu rats which were put in stainless-steel cages at 20 d of age and kept on the diet for 2 additional weeks were not smaller than their +Cu controls. The body weights of male rats following 2 weeks of treatment (+Cu, −Cu) from 20 to 34 d of age, were similar to the body weights of +Cu and −Cu rats derived from dams on the treatment throughout pregnancy and lactation (Table 2).

Regardless of age or duration of treatment, −Cu offspring all showed signs of severe copper deficiency evidenced by marked depressions in serum ceruloplasmin and liver cytochrome oxidase activities (Table 2). The 34-d-old −Cu rats kept on the diet for only 2 weeks had slightly higher ceruloplasmin and liver cytochrome oxidase activities than the 34-d-old −Cu rats that were on the treatment throughout perinatal development. Only one group of −Cu rats (age 20 d) demonstrated any significant decrease in liver GSH-Px activity (Table 2). This decrease in liver

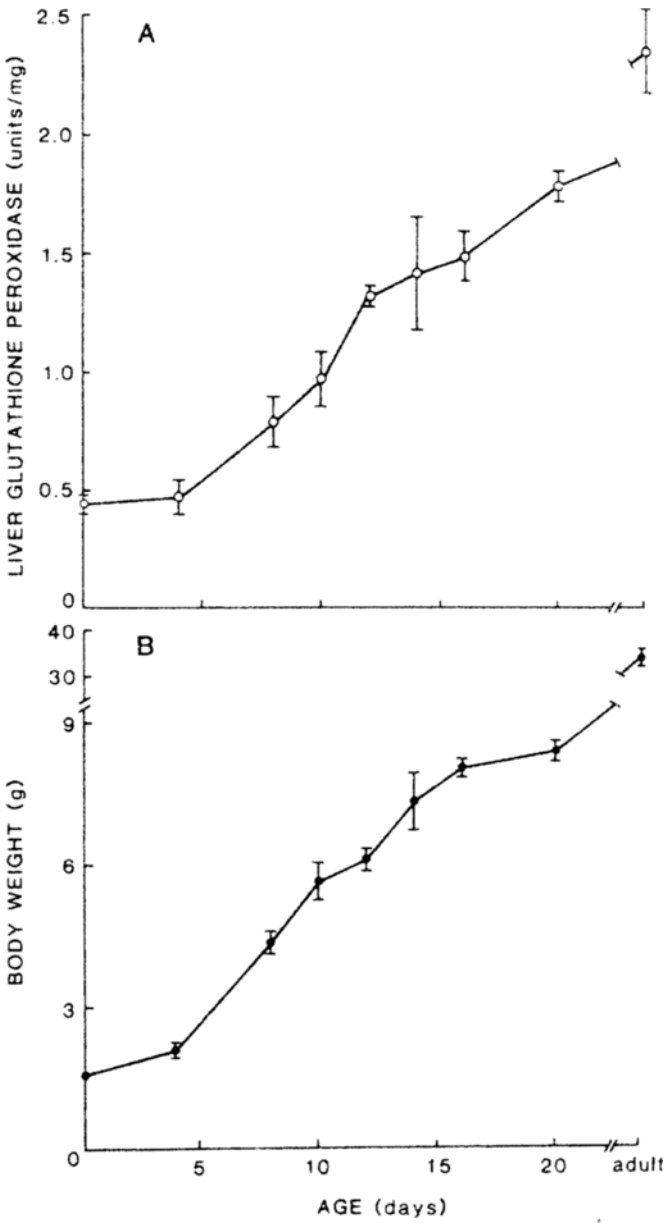

Fig. 1. Development of liver glutathione peroxidase activity (○) and body weight (●) in C57BL mice. Each point represents the mean ± SEM for 5–8 mice. Liver glutathione peroxidase activity was determined spectrophotometrically using *t*-butylhydroperoxide as substrate. Adult mice were 6-month-old males. Dams nursing the suckling mice were fed a nonpurified diet containing about 0.5 ppm Se and 12–14 ppm Cu.

GSH-Px (59%) was in a sample of rats with a significant decrease in body weight (33%). Serum GSH-Px was determined within this same group of rats (+Cu, −Cu) and no mean difference existed: +Cu was 4.36 ± 0.46 (N = 4) and −Cu

TABLE 2
Effect of Dietary and Copper Deficiency in Rats on Body Weight and Liver Enzymes[a]

		Age, days			
	Group	12	20	34	34[b]
Body wt, g	+Cu	29.0 ± 0.9	56.8 ± 0.5	122 ± 8.8	110 ± 13.9
	−Cu	25.0 ± 0.7*	38.3 ± 6.2*	109 ± 21.2	116 ± 17.9
Ceruloplasmin,	+Cu	30.3 ± 3.4	55.7 ± 5.0	86.4 ± 17.7	85.4 ± 9.9
units/L	−Cu	0.12 ± 0.15*	<0.1*	0.29 ± 0.50*	0.68 ± 0.81*
Glutathione					
peroxidase,	+Cu	0.684 ± 0.182	1.73 ± 0.25	1.97 ± 0.17	1.58 ± 0.32
units/mg	−Cu	0.518 ± 0.063	0.712 ± 0.111*	1.70 ± 0.10	1.71 ± 0.53
Cytochrome					
oxidase,	+Cu	0.474 ± 0.041	0.553 ± 0.085	—	0.504 ± 0.038
units/mg	−Cu	0.240 ± 0.010*	0.128 ± 0.049*	0.235 ± 0.017	0.324 ± 0.059*

[a]Copper deficiency was produced during perinatal development by feeding dams a copper-deficient (−Cu) diet during gestation and lactation. Offspring were sampled at the ages indicated. Control offspring were derived from dams which were given copper in their drinking water (+Cu) at 20 ppm. Values are means ±SD of four randomly selected rats from at least two litters; an * indicates the means were different, $P < 0.01$, by Student's t-test.

[b]Rats were maintained on the treatments for two weeks beginning at 20 d of age. Values are means ±SD of 12 male animals from three separate experiments.

was 4.05 ± 0.44 ($N = 4$) units/mL. Younger −Cu rats, age 12 d, had a modest (14%) decrease in body weight, but no drop in liver or serum GSH-Px. The liver and serum GSH-Px values for 12-d-old rats were 2.5 times lower than the corresponding values for +Cu rats that were 20-d-old (unpublished), demonstrating an age-dependent character of GSH-Px in rats as well as in mice.

Discussion

Many factors, nutritional and nonnutritional, influence the level of the selenoenzyme, glutathione peroxidase (GSH-Px), in tissues. Age of the animal has a pronounced effect on liver GSH-Px activity, as was originally shown by Pinto and Bartley (*25*) in rats. Mouse liver also shows this age-dependence (Fig. 1A). Fetal rat GSH-Px activity is similar to newborn levels (*26*). GSH-Px in other tissues, such as blood and brain, does not change as greatly as in liver with age (*23*). Sex of the adult rat also influences liver GSH-Px activity. Female values are about twice that of males (*23, 25*). In contrast, sex had no effect on adult liver GSH-Px in C57BL mice. By far the most prominent factor in determining GSH-Px activity is dietary selenium. In some tissues, such as liver, the activity falls to nondetectable levels following selenium deficiency (*27*). Other dietary factors have also been reported to influence liver, but not erythrocyte, GSH-Px activity such as riboflavin (*28*), iron (*29*) and, most recently, copper (*9, 10*). The studies dealing with riboflavin deficiency in pigs (*28*) and iron deficiency in rats (*29*) are complicated by the

fact that the dietary treatments influenced growth of the animals. Our data in mice and rats (Tables 1, 2) indicate that growth depression is associated with low liver GSH-Px activity.

The relationship between dietary copper and GSH-Px activity is unclear. Data from studies with rats, in which a purified diet containing all known nutrients including supplemental Se, but not Cu, was used, indicate that liver GSH-Px activity is not affected by dietary copper deficiency (*7, 8, 18*). Data with younger animals indicate that liver GSH-Px is altered in −Cu mice and rats and in $Mo^{br/y}$ mice only when a concomitant growth depression exists (Table 1, 2). Growth was not reported in the studies of Balevska et al. (*9*), but most likely the −Cu rats were smaller, since they were fed a diet based on powdered milk (with no mention of an iron supplement) and compared to control rats fed a "standard laboratory diet" containing 15 ppm Cu. Other data argue against a growth depression correlation. In the studies of Jenkinson et al. (*10*), both −Cu and +Cu rats were fed a milk-based diet supplemented with iron. Body weights were said to be comparable, yet the investigators found significant decreases in liver GSH-Px activity in the −Cu rats. Furthermore, in the studies of Paynter and Martin (*8*), body weight was reduced in the −Cu rats but liver GSH-Px was not. Other evidence that suggests that a Se–Cu interaction exists was obtained in a study conducted with sheep concerning copper toxicity (*30*). Accumulation of Cu in liver was associated with an increase in both Se content and GSH-Px activity.

Degree of copper deficiency does not explain the apparent difference in results between investigators since, based on liver SOD depletion, the two groups of −Cu animals used by Jenkinson et al. (*10*) and Paynter and Martin (*8*) were both severely deficient as residual SOD levels were 14 and 17% of control values in the respective studies. Copper deficiency within a tissue is also not a factor, since some −Cu mice have low cytochrome oxidase but normal GSH-Px, while $Mo^{br/y}$ mice have normal cytochrome oxidase but low GSH-Px (Table 1) (*18*). Furthermore, in both −Cu and $Mo^{br/y}$ mice, copper deficiency is much more pronounced in brain tissue than in liver, yet brain GSH-Px is not altered in either −Cu or $Mo^{br/y}$ mice (*21*).

Jenkinson et al. have examined the labeling pattern of [^{75}Se]-selenite in −Cu rats and suggested that the tissue distribution of ^{75}Se was similar to that observed in earlier studies dealing with dietary selenium deficiency (*10*). However, liver seems to be the only tissue studied thus far that demonstrates lower GSH-Px following copper deficiency (Table 2) (*9, 10*). It is hard to explain how liver GSH-Px can be reduced by 60% (Table 2) while serum GSH-Px is normal, if the animals are truly selenium-deficient, since serum GSH-Px has been shown to be a sensitive indicator of selenium status (*27*). In previous studies, GSH-Px has been shown to be normal or elevated in copper-deficient animals in erythrocytes (*7, 8, 11, 13, 18*), brain (*18, 21*), kidney (*18*), and heart (*7, 8*). Lung GSH-Px was not reproducibly altered by dietary copper deficiency (*10*). The tissues that contain lower GSH-Px activity, such as $Mo^{br/y}$ liver (*18*) and the riboflavin-deficient swine liver (*28*), also contain less selenium. The reduced activity of GSH-Px, therefore, results from a lack of selenium rather than from the presence of an endogenous inhibitor (*18*). It is not known how copper or riboflavin deficiency sometimes leads to lower

liver selenium levels. Jenkinson et al. (*10*) did show that −Cu rats had an increased fecal loss of ^{75}Se. This loss was not caused by an enhanced biliary excretion. Other research must be conducted with nutritionally adequate diets to determine if a true copper–selenium interaction exists.

Copper deficiency may predispose cellular organelles, organs, or organisms towards lipid peroxidation, but the etiology of this predisposition is unknown. There exists ample evidence that inadequate dietary copper leads to decreased activity of the cuprozinc protein, superoxide dismutase (SOD), an enzyme that is thought to be involved in the prevention of lipid peroxidation (*1, 2*). Decreases in SOD activity have been established in several tissues of experimental rodents (*3–13*) as well as in erythrocytes of swine (*31*), chickens (*32*), sheep (*33*), and humans (*34*). However, enhanced lipid peroxidation during copper deficiency has only been demonstrated clearly for isolated liver mitochondria (*7, 9, 14*). Many factors may lead to enhanced lipid peroxidation in −Cu liver mitochondria besides low SOD activity, including altered lipid composition (*15*), lowered levels of SH groups (*16*), and increased mitochondrial iron in the outer membrane (*35*). Balevska et al. (*9*) have suggested that failure to metabolize H_2O_2 in −Cu liver mitochondria may explain the enhanced lipid peroxidation. They found decreases in liver GSH-Px and catalase as well as in SOD activity. The activity of manganese–SOD was unaffected (*9*). However, Paynter has found enhanced lipid peroxidation in −Cu rat liver mitochondria without evidence of lower GSH-Px activity (*7*). Paynter feels that SOD or some other copper-dependent factor may be involved. Further work is necessary to determine whether lipid peroxidation is a significant pathological result of copper deficiency and, if so, what factors such as altered SOD, GSH-Px, Fe, unsaturated fatty acids, or SH groups might be responsible.

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References

1. I. Fridovich, *Science* **201,** 875 (1978).
2. B. A. Svingen, F. O. O'Neal, and S. D. Aust, *Photochem. Photobiol.* **28,** 803 (1978).
3. J. R. Prohaska and W. W. Wells, *J. Neurochem.* **23,** 91 (1974).
4. J. R. Prohaska and W. W. Wells, *J. Neurochem.* **25,** 221 (1975).
5. R. F. Morgan and B. L. O'Dell, *J. Neurochem.* **28,** 207 (1977).
6. D. I. Paynter, R. J. Moir, and E. J. Underwood, *J. Nutr.* **109,** 1570 (1979).
7. D. I. Paynter, *Biol. Trace Elem. Res.* **2,** 121 (1980).
8. D. I. Paynter and G. B. Martin, *Biol. Trace Elem. Res.* **2,** 175 (1980).
9. P. S. Balevska, E. M. Russanov, and T. A. Kassabova, *Int. J. Biochem.* **13,** 489 (1981).

10. S. G. Jenkinson, R. A. Lawrence, R. F. Burk, and D. M. Williams, *J. Nutr.* **112,** 197 (1982).
11. W. Bohenkamp and U. Weser, *Biochim. Biophys. Acta* **444,** 396 (1976).
12. W. J. Bettger, T. J. Fish, and B. L. O'Dell, *Proc. Soc. Exp. Biol. Med.* **158,** 279 (1978).
13. E. M. Russanov and T. A. Kassabova, *Int. J. Biochem.* **14,** 321 (1982).
14. E. Russanov and E. Ivancheva, *Acta Physiol. Pharmac. Bulg.* **5,** 67 (1979).
15. C. H. Gallagher and V. E. Reeve, *Aust. J. Exp. Biol. Med. Sci* **49,** 453 (1971).
16. E. A. Ivancheva and E. M. Russanov, *C. R. Acad. Bulg. Sci.* **28,** 975 (1975).
17. D. M. Hunt, *Nature* **249,** 852 (1974).
18. J. R. Prohaska, T. L. Smith, and D. E. Gutsch, in *New Zealand Workshop on Trace Elements in New Zealand,* J. V. Dunckley, ed., University of Otago, Dunedin, New Zealand, 1981, pp. 261–267.
19. Anonymous, *J. Nutr.* **107,** 1340 (1977).
20. J. R. Prohaska, *Nutr. Res.* **1,** 159 (1981).
21. J. R. Prohaska and T. L. Smith, *J. Nutr.* **112,** 1706 (1982).
22. J. R. Prohaska, *Biochim. Biophys. Acta* **611,** 87 (1980).
23. J. R. Prohaska and H. E. Ganther, *J. Neurochem.* **27,** 1379 (1976).
24. M. A. K. Markwell, S. M. Haas, L. L. Bieber, N. E. Tolbert, *Anal. Biochem.* **87,** 206 (1978).
25. R. E. Pinto and W. Bartley, *Biochem. J.* **112,** 109 (1969).
26. I. Mavelli, F. Autuori, L. Dini, A. Spinedi, M. R. Ciriolo, and G. Rotilio, *Biochem. Biophys. Res. Commun.* **102,** 911 (1981).
27. H. E. Ganther, D. G. Hafeman, R. A. Lawrence, R. E. Serfass, and W. G. Hoekstra, in *Trace Elements in Human Health and Disease,* vol. II, A. Prasad, ed., Academic Press, New York, NY, 1976, pp. 165–234.
28. P. S. Brady, L. J. Brady, M. J. Parsons, D. E. Ullrey, and E. R. Miller, *J. Nutr.* **109,** 1615 (1979).
29. Y. H. Lee, D. K. Layman, and R. R. Bell, *J. Nutr.* **111,** 194 (1981).
30. S. R. Gooneratne and J. McC. Howell, in *Trace Element Metabolism in Man and Animals (TEMA-4),* J. McC. Howell, J. M. Gawthorne, and C. L. White, eds., Australian Academy of Science, Canberra, 1981, pp. 468–470.
31. D. M. Williams, R. E. Lynch, G. R. Lee, and G. E. Cartwright, *Proc. Soc. Exp. Biol. Med.* **149,** 534 (1975).
32. W. J. Bettger, J. E. Savage, and B. L. O'Dell, *Nutr. Repts. Intl.* **19,** 893 (1979).
33. K. A. Andrewartha and I. W. Caple, *Res. Vet. Science,* **28,** 101 (1980).
34. S. Okahata, Y. Nishi, S. Hatano, Y. Kobayashi, and T. Usui, *Eur. J. Pediatr.* **134,** 121 (1980).
35. D. M. Williams, F. S. Kennedy, B. G. Green, and J. P. Jordan, *Fed. Proc.* **41,** 460 abs. (1982).