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Superoxide Dismutase Activity in Copper-Deficient Swine[1] (38844)

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Interest in the biologic role of superoxide anion radical has been stimulated by the demonstration that its inactivation is accomplished by an enzymatic disproportionation, $2H^+ + 2O_2^- \rightarrow H_2O_2 + O_2$. In eukaryotes this reaction is catalyzed by a copper–zinc protein which is present both in the cytosol and in the mitochondrial intermembranous space and also by a manganese enzyme which is found in the mitochondrial matrix (1–3). Nutritional experiments confirming that copper is necessary for the activity of the cytoplasmic enzyme have not been published.

The purpose of this investigation was to study the dependence of superoxide dismutase (SOD) activity on dietary copper in pigs and to relate temporally the decline in its activity to that of the plasma copper concentration, the plasma ceruloplasmin concentration, and the volume of packed red cells (VPRC) as copper deficiency progresses.

Materials and Methods. Copper-deficient and control swine were raised on an evaporated milk diet as reported previously (4). Both groups of animals were injected intramuscularly with 2.0 g of elemental iron as iron–dextrin (Pig-Dex) to ensure adequate iron stores.

Hematologic determinations were carried out by standard methods (5). The plasma copper concentration was measured colorimetrically with oxalyldihydrazide (6); the ceruloplasmin concentration was assayed by the paraphenylenediamine oxidase method of Ravin (7); and protein concentrations were estimated by the method of Lowry (8). Superoxide dismutase activity was assayed at pH 7.8 by its inhibition of the reduction of cytochrome *c* by superoxide anion which was generated by xanthine oxidase (9). Subcellular fractions were obtained by differential centrifugation in unbuffered 0.25 *M* sucrose using the method of Schneider (10) and assayed for superoxide dismutase activity after extraction by a modification of the method of Tsuchihashi (11) using 2 vol of starting material. Inhibition studies of mitochondrial superoxide dismutase activity were performed in 0.001 *M* KCN (12).

Results. Blood analyses were first performed 8 wk after the beginning of the copper-deficient diet (Fig. 1). By this time the plasma copper concentration was 5–10% of the value in littermate control pigs and the concentration of copper stabilized at this level. The ceruloplasmin concentration at 8 wk was 8% of the control value and decreased further to less than 1% of the control value by 12 wk on the diet. The red cell SOD activity declined more slowly than the ceruloplasmin, being 38% of the control value at 8 wk and 12% by 15 wk. The red cell SOD activity decreased to 35% of the control value before the development of significant anemia (Table I) and continued to decrease as the anemia increased in severity (Table I and Fig. 1).

The SOD activity in the liver homogenates in the cytosol and mitochondrial fractions decreased, respectively, to 5%, 8%, and 16% of control values (Table II). The cuprozinc SOD is inhibited by cyanide while the manganoenzyme is not (2). The mitochondrial cyanide-sensitive SOD decreased in copper deficiency to a greater extent than the cyanide-insensitive SOD (Table III).

Discussion. The SOD activity of both red cells and liver decreased in the copper-deficient animals. The decrease in red cell SOD activity preceded the development of anemia but the red cell SOD activity was not depleted as rapidly or to as great a degree as the plasma copper or ceruloplasmin. This is understandable since the turnover rate of red cells is slower than that of ceruloplasmin.

The amount of SOD inside cells greatly exceeds the steady-state concentration of superoxide. Whether 5–15% of the normal

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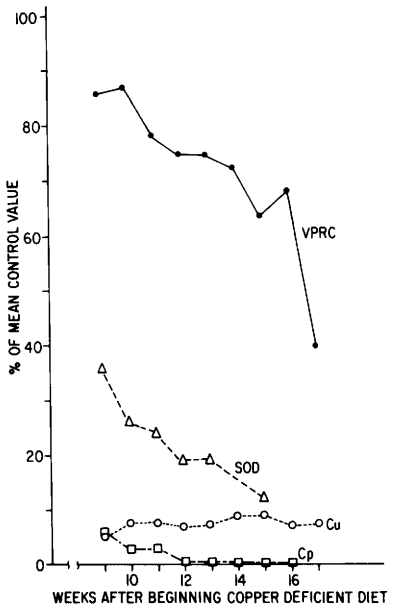

FIG. 1. Changes in superoxide dismutase activity (SOD), plasma copper concentration (Cu), plasma ceruloplasmin concentration (Cp), and the volume of packed red cells (VPRC), during the progression of copper deficiency. Each point represents the mean of seven copper-deficient animals expressed as a percentage of the mean value in littermate, copper-replete controls.

TABLE I. RED BLOOD CELL SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN RELATION TO THE DEGREE OF ANEMIA.

	V.P.R.C.[a] (ml/100 ml)	SOD activity[b] (units/ml RBC[b])
Control	40–45	559 ± 18.3
Copper deficient	40–45	191 ± 16.3
Copper deficient	30–40	147 ± 13.4
Copper deficient	20–30	77 ± 9.5

[a] V.P.R.C. refers to volume of packed red cells.
[b] The values refer to mean ± 1 SE for at least 24 determinations.

activity is sufficient to protect cells from the ravages of free radicals under normal conditions is not known. It has been observed that the survival time of copper-deficient erythrocytes is shortened (13), the osmotic fragility of such cells is increased, and the membrane is excessively permeable to sodium (14). Whether these changes are a consequence of membrane damage due to

TABLE II. SUPEROXIDE DISMUTASE SPECIFIC ACTIVITY IN SUBCELLULAR FRACTIONS OF LIVER FROM CONTROL AND COPPER-DEFICIENT SWINE.

		Superoxide dismutase activity (U/mg protein)		
	N	Liver	Cytosol	Mitochondria
Control	15	12.3[a] ±1.12	17.0 ±1.61	2.5 ±0.33
Copper deficient	10	0.6 ±0.14	1.4 ±0.28	0.4 ±0.19

[a] Values represent mean ± 1 SE. All differences between control and deficient values significant at .001 level.

TABLE III. CYANIDE-SENSITIVE AND CYANIDE-INSENSITIVE SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN MITOCHONDRIA FROM CONTROL AND COPPER-DEFICIENT SWINE.

Group	Cyanide Sensitive (units/ml RBC)	Cyanide Insensitive (units/ml RBC)
Control	1.68	0.53
	1.13	0.43
	1.18	0.38
Deficient	0.19	0.44
	0.00	0.33
	0.27	0.27

superoxide anion brought about by the SOD enzyme deficiency is speculative at this time. However, erythrocytes from swine severely deficient in copper should provide a system to study such effects.

Summary. These experiments demonstrate the dependency of cuprozinc superoxide dismutase activity in red cells and liver on an adequate dietary intake of copper. The superoxide dismutase activity in red cells decreased to 15% of control values and, therefore, these cells may be used as a convenient model for studying the physiologic consequences of free radicals.

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