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Uptake of ^{67}Cu by Ceruloplasmin *In Vitro* (38878)

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Scheinberg and Morell (1) clearly showed that at physiologic pH there was no exchange between the copper in ceruloplasmin and that in the surrounding solution, either in vivo or in vitro. However, by decreasing the pH and adding ascorbic acid, exchange could be induced.

Joselow and Dawson (2, 3) effected an exchange between environmental copper (^{64}Cu) and ascorbate oxidase copper at pH 6 only when ascorbic acid was added. Similarly, Dressler and Dawson (4) were able to induce copper exchange with tyrosinase, but again only when a substrate for the enzyme was furnished.

After confirming that ^{67}Cu could be taken up by tyrosinase when L-tyrosyl-L-alanine was added at about pH 7, it was decided to try to induce an exchange between ceruloplasmin and ^{67}Cu, at neutral pH, by adding *p*-phenylenediamine, a substrate for this enzyme.

Methods. Human ceruloplasmin (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.1 *M* Tris buffer, pH 7.2, to a concentration of 30 units/ml. *p*-Phenylenediamine dihydrochloride (PPD) was dissolved in copper-free water, 10 mg/10 ml. $^{67}CuCl_2$, carrier-free, from Oak Ridge (TN), was diluted in water to about 0.25 μCi/ml. The final reaction mixture contained 3.2 ml: 2.0 ml of $^{67}Cu^{2+}$ solution, 1.0 ml of Tris buffer, 0.1 ml of enzyme, and 0.1 ml of PPD. Three blanks lacked enzyme and PPD, enzyme, or PPD. After 1 hr of incubation at 37°, the reaction mixture was mixed for 1 hr, with shaking, with 2 g of Chelex-100 (Bio-Rad Laboratories, Richmond, CA), a procedure that removed well over 99% of $^{67}Cu^{2+}$ from an aqueous solution of $^{67}CuCl_2$. One hour was selected for incubation because no further exchange occurred thereafter. When ceruloplasmin was incubated with $^{67}Cu^{2+}$ in the absence of PPD, all but 1.5–2% of the $^{67}Cu^{2+}$ was free to be chelated by the resin.

Results. In Fig. 1 are shown net uptakes of $^{67}Cu^{2+}$ by ceruloplasmin at various pH values achieved by changing the pH of the Tris buffer. Approximately 70% of the added $^{67}Cu^{2+}$ was taken up by the ceruloplasmin between pH 6 and pH 7.5, but uptake fell off sharply as the pH dropped below 6. After the incubation, when the ceruloplasmin was passed through a column of Sephadex G-100 in 0.1 *M* Tris buffer, pH 6.8, the radioactivity and the PPD-oxidasic activity emerged together in the void volume, distinct from $^{67}Cu^{2+}$ which came out at $V_E/V_O = 3$. There was no decrease in ceruloplasminic activity.

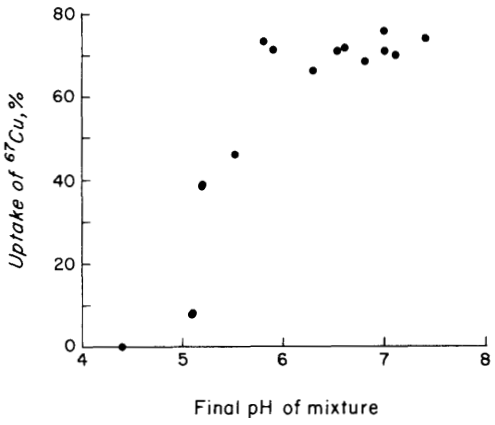

FIG. 1. Uptake of $^{67}Cu^{2+}$ by human ceruloplasmin when carrier-free $^{67}CuCl_2$ and *p*-phenylenediamine were incubated for 1 hr at 37°. The mixture was buffered with Tris at various pH values.

The addition of stable copper (aqueous solution of $CuSO_4$) to the ceruloplasmin–PPD–$^{67}Cu^{2+}$ incubation mixture decreased the percentage uptake of $^{67}Cu^{2+}$ by the ceruloplasmin (Fig. 2).

Discussion. The observation that ceruloplasmin can exchange its copper for ionic copper at physiologic pH when enzymatically active suggests a biologic role for this enzyme. It may act as a copper donor, but only when it is activated. How this fits with the one known function of ceruloplasmin—mobilization of iron stores—is obscure.

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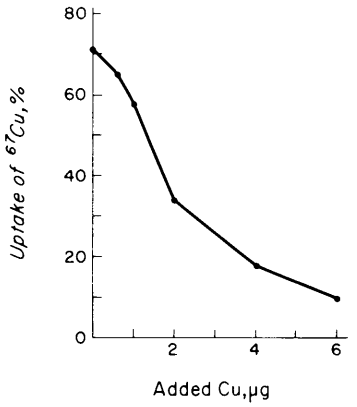

FIG. 2. Effect of added copper (as $CuSO_4$) on uptake of $^{67}Cu^{2+}$ by human ceruloplasmin in the presence of *p*-phenylenediamine. There was 0.3 μg of copper in the ceruloplasmin in the reaction mixtures.

However, virtually all organs can take up radiocopper both from ionic copper and from ceruloplasmin sources, and some even prefer the latter, particularly in copper-deficiency states (5–7). It seems unlikely that all tissues take up copper simply to mobilize iron. A more reasonable explanation is the need of other copper-containing enzymes (for example, cytochrome oxidase, amine oxidases, uricase) to acquire their copper (8).

Summary. Solutions of human ceruloplasmin, in Tris buffer at pH values between 6 and 7.5, readily took up carrier-free $^{67}Cu^{2+}$, but only when the substrate, *p*-phenylenediamine, was added.

1. Scheinberg, I. H., and Morell, A. G., J. Clin. Invest. **36,** 1193 (1957).
2. Joselow, M., and Dawson, C. R., J. Biol. Chem. **191,** 1 (1951).
3. Joselow, M., and Dawson, C. R., J. Biol. Chem. **191,** 11 (1951).
4. Dressler, H., and Dawson, C. R., Biochim. Biophys. Acta **45,** 508 (1960).
5. Owen, C. A., Jr., Amer. J. Physiol. **209,** 900 (1965).
6. Owen, C. A., Jr., Amer. J. Physiol. **221,** 1722 (1971).
7. Terao, T., and Owen, C. A., Jr., Mayo Clin. Proc. **49,** 376 (1974).
8. Owen, C. A., Jr., Fed. Proc. **33,** 667 (1974).

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