Turnover of the Copper and Protein Moieties of Ceruloplasmin

Approximately 95 per cent of the plasma copper in both mouse and man is normally present as a moiety of ceruloplasmin[1]. Ceruloplasmin, an α-globulin containing 0·34 per cent copper, exhibits oxidase activity *in vitro* with a variety of substrates[2] and reversibly releases at least half its copper upon reduction[3]. Whether ceruloplasmin plays a part in the transport of copper or acts as an oxidase *in vivo*, however, is not known. In the work reported here, the function of ceruloplasmin in mice was investigated by determining the rate of exchange of its copper moiety.

Human ceruloplasmin isolated from pooled plasma[3] was kindly supplied by Dr. I. H. Scheinberg. The protein moiety of an aliquot of the preparation was labelled with iodine-131 at *p*H 6·8 using the nitrite method described elsewhere[4]. Iodination did not alter the reactivity of the ceruloplasmin with specific rabbit antisera[5] or change its visible spectrum. More than 98 per cent of the radioactivity of the preparation was precipitable in 10 per cent trichloracetic acid. The iodoceruloplasmin was finally screened in Swiss albino mice for 24 hr. to remove any denatured labelled protein, as suggested by McFarlane[6].

Additional aliquots of human ceruloplasmin were labelled with copper-64 using an exchange method[3]. Of the total radioactivity in these preparations, 92–95 per cent was precipitable with rabbit antiserum.

Labelled mouse ceruloplasmin was obtained by injecting mice intraperitoneally with 100 μgm. copper containing 300 μc. copper-64. Serum collected 24 hr. later had a final activity of 1·7 μc. per ml., of which almost 98 per cent was associated with ceruloplasmin electrophoretically. The pooled serum was used as the preparation of mouse ceruloplasmin in these experiments.

Groups of Swiss albino mice 6–7 weeks old were given 0·5 μc. of one of the labelled ceruloplasmins intravenously; this was equivalent to 20 μgm. human ceruloplasmin labelled with iodine-131, 200 μgm. human ceruloplasmin labelled with copper-64, or 40 μgm. mouse ceruloplasmin labelled with copper-64. The mice were assayed in a well-type sodium iodide crystal scintillator. Three or four mice from each group were killed at intervals; serum and various organs were assayed for radioactivity.

The half-life of iodine-labelled human ceruloplasmin, $t_{i\frac{1}{2}}$, as determined by its disappearance from the total body or from serum (Fig. 1), was 0·92 day. The copper-64 in human and mouse ceruloplasmins disappeared from serum with half-lives, $t_{h\frac{1}{2}}$ and $t_{m\frac{1}{2}}$, of 0·75 and 0·85 days, respectively. With human ceruloplasmin at least, copper-64 disappeared more rapidly than the protein moiety was degraded, suggesting a slow exchange of ceruloplasmin copper in the serum; the half-life of this exchange, $t_{\frac{1}{2}}$, was calculated:

$$t_{\frac{1}{2}} = \frac{t_{i\frac{1}{2}} \cdot t_{h\frac{1}{2}}}{t_{i\frac{1}{2}} - t_{h\frac{1}{2}}} = 4{\cdot}1 \text{ days}$$

From 2 hr. following the injection of labelled human ceruloplasmin to the end of the experiment, less than 1 per cent of the serum radioiodide and less than 2 per cent of the serum radiocopper were not precipitable with rabbit antiserum versus human ceruloplasmin[5].

It can be seen from Fig. 1 that the amount of ceruloplasmin in the total body after distribution

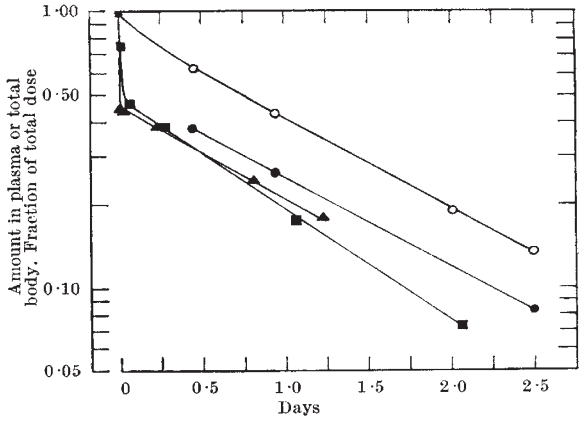

Fig. 1. The disappearance of labelled ceruloplasmins after intravenous injection in mice. From total body: human ceruloplasmin labelled with iodine-131 (○). From plasma: human ceruloplasmin labelled with iodine-131 (●); human ceruloplasmin labelled with copper-64 (■); mouse ceruloplasmin labelled with copper-64 (▲)

of the protein in the body fluids was approximately twice the amount present in the serum. Since the average serum ceruloplasmin concentration in these mice was 13 mgm. per 100 ml. serum and the plasma volume was 1·14 ml., the total ceruloplasmin pool was 0·3 mgm. containing 1·0 μgm. copper. With a half-life of 4·1 days, the amount of copper exchanged within the ceruloplasmin pool per day was 0·17 μgm. The mice studied here ingested 45–75 μgm. copper a day in the diet they received; of this, tracer studies with copper-64 indicated that they absorbed a minimum of 4·5–7·5 μgm. copper a day[7]. It is clear that the amount of ceruloplasmin copper exchanged per day was less than 4 per cent of the amount of copper absorbed from the intestinal tract. This would appear to militate against the suggestion that absorption of copper from the intestines is inhibited or controlled by extensive dissociation of ceruloplasmin copper in the intestinal plasma[3]. The small exchange of ceruloplasmin copper would also indicate that ceruloplasmin does not function to transport copper from the intestines unless the intestines synthesize ceruloplasmin. But even the synthesis of ceruloplasmin by one tissue with subsequent release of its copper in other tissues through degradation could result in the transport of only 1·5 μgm. copper per day.

On the other hand, the observed exchange in ceruloplasmin copper, although quite small, suggests that ceruloplasmin may act, at least in part, as an oxidase *in vivo*.

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