THE DETERMINATION OF CERULOPLASMIN

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In 1948, Holmberg and Laurell[1] isolated from human serum the blue copper-containing α-globulin ceruloplasmin. The molecular weight of this protein is approximately 151,000, and the copper content 0·34 per cent, which corresponds to eight atoms of copper per molecule. Ceruloplasmin possesses *in vitro* oxidase activity towards, for example, epinephrine[2,3], 5-hydroxytryptamine[4], dihydroxyphenylalanine[2], *p*-phenylenediamine[5] and *N*,*N*-dimethyl-*p*-phenylenediamine (DPP)[6].

The present view is that 90–95 per cent of serum copper is tightly bound in ceruloplasmin[7,8]. This is based principally on the fact that variations in copper and ceruloplasmin concentrations are almost always in step with one another. It is thus conceivable that the enzyme plays an important rôle in copper metabolism. The exact nature of its function has not yet been elucidated, however.

It has been reported that the concentrations of copper and of ceruloplasmin in serum are greatly increased in late pregnancy and in many diseases, *e.g.* bacterial infections, neurological disorders, cirrhosis, hepatitis and acute and chronic leukaemia[7]; while in Wilson's disease the concentration of ceruloplasmin is greatly reduced.

Åkersfeldt's report[6] of an increased ceruloplasmin concentration in the sera of patients with mental diseases aroused great interest. Some investigators were able to reproduce Åkersfeldt's results, but many others were not. In our opinion, however, the methods used, all based on the measurement of the oxidase activity of serum, were not optimal. With a modified method, we have also been unable to find any deviation from normal as described by Åkersfeldt. However, a positive correlation was found between age and ceruloplasmin concentration. Our material comprised subjects whose ages ranged from 20 to 60 years[9].

In another series of experiments, a positive correlation between age and total copper in serum was established, as would be expected on the basis of the above-mentioned relationship. The concentration in females was significantly higher than in males for all ages. Again our experiments were carried out with subjects in the age group from 20 to 60 years[10].

It is generally accepted that ceruloplasmin is the only oxidase in human serum, and that ceruloplasmin copper accounts for all tightly-bound serum copper. Deutsch recently published a direct method for the measurement of ceruloplasmin[11]. This prompted us to test these concepts. The first results of these experiments are reported here.

Oxidase activity, ceruloplasmin and total copper were determined in sera obtained from normal males and male patients with a variety of clinical conditions after overnight fasting. For the measurement of oxidase activity,

2·4 ml of 0·12 M Sörensen's buffer solution (pH 6·2) and 0·1 ml of a 4·8% solution of the dihydrochloride of DPP were added to 0·5 ml of serum in a 1 cm cell. The optical density at 552 mμ was read every minute for at least 6 min against a blank which also contained 10 mg sodium azide. The sample was kept at a constant temperature of 25 ±0·05°. Serum copper was determined according to the method described by Rice [12]. The method for the direct measurement of ceruloplasmin[11] was based on the isolation of ceruloplasmin by chromatography on DEAE-cellulose (Serva, Heidelberg, Germany). The optical density of the fraction containing ceruloplasmin was measured at 610 mμ before and after the addition of ascorbic acid. We modified this technique in some respects. The sera were desalted by gel-filtration on Sephadex G-25 (Pharmacia, Uppsala, Sweden) instead of by dialysis. The DEAE-cellulose column was first eluted with 0·05 M acetate

Table 1

1	2	3	4	5	6	7
Subject	*Age* (years)	*DPP-oxidase activity* ($\Delta E.10^3$/min)*	*Ceruloplasmin* (mg/100ml)†*	*Ceruloplasmin-copper (calc.)* (μg/100ml)	*Total copper* (μg/100ml)*	$\frac{\textit{Ceruloplasmin-copper} \times 100}{\textit{Total copper}}$ (%)
1	19	97	38·0	129	152	85
2	20	88	27·5	93	109	85
3	18	67	18·0	61	100	61
4	20	105	34·0	115	129	89
5	50	82	31·0	105	142	74
6	36	75	21·0	72	109	66
7	19	79	22·0	75	95	79
8	20	54	21·5	73	99	74
9	24	55	20·5	69	81	85
10	40	85	31·0	106	134	79
11	20	57	19·5	67	97	69
12	20	62	22·5	76	97	78
13	23	55	21·0	72	82	88
14	20	45	17·5	60	85	71
15	35	79	29·0	99	121	82
16	51	75	21·0	72	107	67
17	19	77	24·5	83	112	74
18	18	103	29·5	101	151	67
19	53	61	20·5	69	108	64
20	41	81	28·5	97	124	78
21	51	66	22·5	77	117	66
22	41	55	17·5	60	105	57
23	38	72	25·5	86	118	73
24	44	73	23·5	80	110	73
25	38	69	24·0	81	113	72
26	53	61	18·0	61	106	58
27	19	91	34·5	118	134	88
28	53	66	25·0	85	117	73
29	19	50	18·5	63	82	77
30	38	50	18·5	63	90	70
31	35	62	21·0	71	106	67
32	39	76	22·0	74	112	66
33	—	73	23·0	78	108	72
Average	± s. d.	71 ± 15	24·0 ± 5·0	82 ± 17	110 ± 18	74 ± 9

* Average from duplicates. † 5 ml of serum was used for each determination.

buffer solution (pH 5·5) containing 0·05 M instead of 0·1 M sodium chloride as in the original procedure. Lastly, we added 1 mg of ascorbic acid crystals instead of 0·1 ml. of a 1% ascorbic acid solution in acetate buffer solution (pH 5·5). Except for the spectrophotometric determination, all manipulations were performed in a cold room at 4°.

The results are summarized in *Table 1*. As can be seen from columns 3 and 4, there is a good agreement between the ceruloplasmin content and the DPP-oxidase activity. In accordance with this, a strong positive correlation ($r = 0{\cdot}86$) was calculated.

The figures in column 7 show that the copper bound in ceruloplasmin accounts for only 57–89 per cent (average 74 per cent) of the serum copper. This is considerably lower than the values reported in the literature for bound copper[7,8].

Several explanations are possible for this discrepancy. The most likely are, in our opinion, the following:

(1) The values obtained by the method of Deutsch are too low. In this connection, the existence of several ceruloplasmin fractions in human serum is of interest[13,14].

(2) Another copper-containing protein exists in serum.

Some arguments can be given against the first view: (a) The reproducibility of the method is good; the standard deviation calculated from 33 duplicate measurements is 0·9 mg/100ml. (b) Variations in ionic strength (sodium chloride concentration increased up to 1·0 M) and pH (pH 5·0–6·0) failed to give higher results. (c) Deutsch could not find, by immunological means, any ceruloplasmin other than that in the ceruloplasmin-containing fraction[15]. (d) From reports in the literature describing the determination of ceruloplasmin by means of a direct method (immunologically[16], by direct spectrophotometry[17] or by spectrophotometry after DEAE-cellulose chromatography[15]), the same discrepancy between tightly-bound copper and ceruloplasmin copper can be calculated.

To summarize, we think firstly that the measurement of DPP-oxidase activity in serum provides a reliable estimate of the concentration of ceruloplasmin; and secondly that the equivalence of ceruloplasmin and tightly-bound serum copper is doubtful.

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