Significance of plasma copper and caeruloplasmin concentrations in rheumatoid arthritis

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Bajpayee, D. P. (1975). Annals of the Rheumatic Diseases, 34, 162. Significance of plasma copper and caeruloplasmin concentrations in rheumatoid arthritis. To understand the role of copper in initiating protein alterations in rheumatoid arthritis (RA) as reported previously, concentrations of copper and caeruloplasmin were determined in RA patients. The mean copper concentration of the RA population examined was 24.8 μmol/l (157.5 μg/100 ml), and the mean caeruloplasmin concentration in this RA population was 45.52 mg/100 ml. These values are not different from those reported by previous workers. However, when the RA population was divided into three groups according to sex and oestrogen therapy it was found that caeruloplasmin and copper concentrations in the group of female RA patients on oestrogens was significantly different from other groups (P < 0.001). A highly significant (P < 0.01) positive correlation was obtained between copper and caeruloplasmin concentrations (r = 0.91).

Concentrations of copper and caeruloplasmin failed to explain the low sulphydryl content of plasma which was observed to be independent of these two parameters. Increased α2-globulin concentration, which was refractory to chrysotherapy but 'finger-printed' with a pure preparation of caeruloplasmin in electrophoresis, along with the absence of Kayser-Fleischer rings, supports the contention that copper is not present in a free ionic state in RA patients. This study shows that only a concurrent oestrogen therapy raises copper and caeruloplasmin concentration significantly in a female RA population. Past investigators appear to have overlooked this fact, and it could be that a disproportionate sex distribution (more female rheumatoid arthritics) could cause misleading results in RA studies. The role of oestrogens, copper, and caeruloplasmin in causing exacerbation of RA symptoms is discussed.

This investigation was designed to study plasma copper and caeruloplasmin concentration in rheumatoid arthritis (RA) and to collect data that might prove valuable in understanding the factors causing protein disorders in this disease as mentioned by Lorber, Pearson, Meredith, and Gantz-Mandell (1964). Numerous recent reports have indicated that plasma copper and caeruloplasmin levels are increased in concentration in RA. For instance, Niedermeier and Griggs (1971) have pointed to the fact that mean values of serum copper are significantly higher in the RA population as compared to the normal population. Plantin and Strandberg (1965), using neutron activation analysis, have shown that mean whole blood concentration of copper in RA was considerably raised over that found in a normal population. These studies have reported only overall mean values of copper concentration in RA populations investigated. This has masked the true nature of the data by overlooking the fact that any RA population under study will have a disproportionate sex distribution (female to male 2:1) which could cause misleading interpretation of results. Considering the evidence that caeruloplasmin and copper levels are raised in females taking synthetic oestrogens (Carruthers, Hobbs, and Warren, 1966) and considering the growing contention that oestrogens are implicated in inducing rheumatic symptoms (Kay and Bole, 1971), it was felt that dividing the RA population by sex and oestrogen therapy was essential for rational interpretation of copper and caeruloplasmin concentration data and their relationship to disease activity in RA. The effect of the rise in these two parameters

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on plasma sulphydryl group content has been of
further interest and was undertaken to resolve some
questions and extend some observations of previous
studies carried out by Lorber and co-workers
(Lorber, Cutler, and Chang, 1968; Lorber and others,
1964).

**Materials and methods**

**Collection of blood specimens**

Blood was collected into heparinized containers from
patients who had a diagnosis of definite or classical RA,
and normal healthy volunteers of both sexes. The tubes
were centrifuged at 2000 r.p.m. to obtain plasma. Plasma
was either analysed fresh or was frozen at −20°C till
analysed.

**Analysis of copper**

Copper was determined in plasma samples by atomic
absorption spectrometry using internal standards. The
method followed was that described by Lorber and others
(1968) except that the standard solution used in this
laboratory is a cocktail containing copper and gold
at a concentration of 78·7 μmol/l (500 μg/100 ml) and 25·4
μmol/l (500 μg/100 ml), respectively. This mixed standard
enables us to utilize the same plasma sample for both
copper and gold determinations (for patients on chryso-
therapy) by switching to hollow cathode ray tube of the
particular metal to be analysed in the sample. All analyses
were carried out on Model Dial-Atom atomic absorption
spectrometer (Jarrel Ash Division, Fisher Scientific Co.)
at 321 μ wavelength.

**Sulphydryl group analysis**

Sulphydryl groups were determined in plasma samples by
the spectrophotometric method of Ellman (1958). The
plasma samples of twenty healthy, normal individuals of
both sexes analysed for sulphydryl groups by this method
gave a range of 448–734 μmol/l with a mean value of 591
μmol/l.

**Determination of caeruloplasmin**

Caeruloplasmin was determined in plasma samples by the
method outlined by Henry and co-workers (Henry, Chiamori,
Jacobs, and Segalove, 1960). Plasma samples
with high caeruloplasmin concentration were diluted
1:1 with normal saline before enzyme assay.

**Statistical analysis**

Correlation coefficient and linear regression analyses were
carried out as described by Li (1961) to obtain values of r,
and of the estimated regression equations \( \hat{Y}_x = a + b (x - 8) \). In this equation, a, b, and \( \hat{Y}_x \) denote the sample
estimates of \( \alpha \), \( \beta \), and \( \mu \), respectively, of the classical
regression equation \( \mu_{y,x} = \alpha + \beta (x - 8) \). Analysis of
variance was used to test the hypothesis that mean values
of caeruloplasmin and copper in the groups (male RA;
female RA; female RA on oestrogens; normal males;
normal females) are equal.

**Results**

Fig. 1 shows the relationship of copper and caerulo-
plasmin concentration on 44 RA patients. The mean
values for copper and caeruloplasmin of these RA
patients were 24·8 μmol/l (157·5 μg/100 ml) and 45·52
mg/100 ml, respectively. A highly significant cor-
relation was observed between copper and caerulo-
plasmin concentration \( (r = 0·91; \ P < 0·01) \). The
mean copper values in this survey are not different from
those reported by Lorber and others (1968) and
Plantin and Strandberg (1965). The significant
correlation of copper and caeruloplasmin found in
this study is supported by findings of Antonopoulou,
Maragos, Besos, Trichopoulos, Sfikakis, and Daikos
(1971). The Table gives mean values of copper and
cauruloplasmin in RA patients grouped by sex and
oestrogen intake. Analysis of variance shows that the

**Table Mean copper and caeruloplasmin values in normals and in RA patients grouped according to sex and oestrogen intake**

<table>
<thead>
<tr>
<th></th>
<th>No. patients</th>
<th>Caeruloplasmin (mg/100 ml)</th>
<th>Copper (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male RA patients</td>
<td>14</td>
<td>26·28 (20–34)*</td>
<td>15·4 (12·4–19·7)*</td>
</tr>
<tr>
<td>Female RA patients</td>
<td>14</td>
<td>31·28 (20–44)</td>
<td>17·3 (11·8–23·6)</td>
</tr>
<tr>
<td>Female RA patients on oestrogens</td>
<td>19</td>
<td>59·37 (43–74)†</td>
<td>32·6 (29·3–43)†</td>
</tr>
<tr>
<td>Normal males</td>
<td>9</td>
<td>27·4 (18–52)</td>
<td>15·9 (11·8–27·5)</td>
</tr>
<tr>
<td>Normal females</td>
<td>13</td>
<td>30·55 (20–47)</td>
<td>17·3 (11·8–23·6)</td>
</tr>
</tbody>
</table>

* Data in parentheses refer to range.
† Highly significantly (P < 0·001) different from other groups.
patient on oral contraceptive consistently maintained a high copper and caeruloplasmin level with raised \( \alpha_2 \)-globulin. However, the copper and caeruloplasmin content remained normal and the \( \alpha_2 \)-globulin dropped under the influence of chrysotherapy in the patient not taking any oestrogens. The \( \alpha_2 \)-globulin present in the patient taking oral contraceptive migrated and ‘fingerprinted’ exactly with a pure commercial caeruloplasmin preparation (Sigma Chemical Co., St. Louis, Mo.) on electrophoresis. These findings are similar to those reported by Bearn and Kunkel (1954), Gubler, Lahey, Brown, Smith, Cartwright, and Wintrobe (1951), and Scheinberg and Morell (1957).

**Discussion**

Numerous reports have established the role of both endogenous and exogenous oestrogens in raising the concentration of serum copper and caeruloplasmin (O’Leary and Spellacy, 1969; O’Leary, 1969; O’Leary and Feldman, 1968; Carruthers and others, 1966) in normal females. Oestrogens also produce hypergammaglobulinaemia, increase the number of plasma cells, and produce stromal hypertrophy of spleen in NZB/NZW mice (Walker and Bole, 1971). Oral contraceptives have been shown to initiate antinuclear antibody production, raise the rheumatoid factor titre, and produce a positive LE cell test (Kay and Bole, 1971). Past evidence (Nicol, Vernon-Roberts, and Quantock, 1966a, b; Nicol, Bilbey, Charles, Cordingley, and Vernon-Roberts, 1964) suggests that oestrogens are also highly potent in stimulating the reticuloendothelial system (RES) and in raising body defense. These serological abnormalities induced by oestrogens in RA patients cannot explain the raised levels of copper and caeruloplasmin which are also found in normal

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**FIG. 2** Typical levels of copper, sulphydryl, and \( \alpha_2 \)-globulin in a female patient (Case 1) on oral contraceptive containing synthetic oestrogen and a female patient on no oestrogens (Case 2). The numbers above the copper peaks show the caeruloplasmin concentration of the sample.

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**FIG. 3** Pathway showing possible relationship of oestrogens in exacerbation of RA symptoms. RES, reticuloendothelial system. * Evidence already present in literature, see text.
human females taking oral contraceptives. The lack of correlation found between copper and sulphhydril levels in Fig. 2 support the contention that increased caeruloplasmin contributes essentially to the total increase in copper concentration. This copper is not available in the free state to catalyse the oxidation of sulphhydril linkage of proteins (Saroff and Choate, 1958). Additional support that copper is not present in a free state comes from the absence of Kayser-Fleischer rings in RA patients with high copper levels. In contrast, in Wilson’s disease Kayser-Fleischer rings are present in 92% of the cases and the presence of free copper is well established (Cumings, 1971).

Our study fails to confirm the report of Lorber and others (1968) that an error is involved in copper metabolism in RA. However, it shows the specificity of oestrogens in raising caeruloplasmin levels in female RA patients. Fig. 3 presents a scheme through which oestrogens may exacerbate but not produce the symptoms of RA on the basis of evidence cited and the findings of this study. This study shows that copper and sulphhydril estimations are not meaningful parameters to explain the protein disorders found in RA.

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