

Ceruloplasmin as an Indicator of Copper Status in Cattle and Sheep

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ABSTRACT

The relationship between ceruloplasmin, a metalloenzyme with oxidase activity, and copper was investigated in cattle and sheep. The oxidase activity of ceruloplasmin correlated closely with the serum or plasma copper concentrations in cattle. The respective correlation coefficients were 0.83 and 0.60. In sheep serum, a correlation coefficient of 0.92 was obtained. In each instance, the relationship remained linear from the deficient to the high normal ranges of copper. Comparison of the linear regression relationships indicated the ceruloplasmin activity in bovine serum was statistically lower than the activity in bovine plasma ($P < 0.0001$), though the intercepts from the regression lines of the two relationships were similar ($P = 0.412$). Comparisons of ovine and bovine serum-ceruloplasmin relationships indicated that a significant species difference was present. Ovine ceruloplasmin activity increased more rapidly as compared to the corresponding bovine activity over the range of copper concentrations investigated ($P < 0.0001$). The intercept from the ovine regression relationship was also lower ($P < 0.0001$). A correlation coefficient of 0.35 was observed between the serum ceruloplasmin activity and hepatic copper concentrations in cattle indicating that the mathematical relationship was not as well defined. Ceruloplasmin activity appears to correlate more closely with serum or plasma copper concentrations as compared to corresponding liver copper concentrations.

Key words: Copper deficiency, ceruloplasmin, metalloenzyme, oxidase activity, cattle, sheep.

RÉSUMÉ

Cette expérience consistait à déterminer, chez les bovins et les moutons, la relation entre la céruloplasmine, un metalloenzyme doué d'une activité oxydasique, et le cuivre. L'activité oxydasique de la céruloplasmine afficha une relation étroite avec la teneur en cuivre sérique ou plasmatique, chez les bovins, comme le démontrèrent des coefficients de corrélation respectifs de 0,83 et 0,6. Le coefficient de corrélation relatif au sérum des moutons se situait par ailleurs à 0,92. Dans chaque cas, la relation demeura linéaire, à partir des taux de cuivre inférieurs à la normale jusqu'aux taux normaux élevés. La comparaison des relations de la régression linéaire révéla que l'activité de la céruloplasmine était statistiquement moins élevée ($P < 0,0001$) dans le sérum que dans le plasma des bovins, même si les intercepts des lignes de régression des deux relations s'avéraient semblables ($P = 0,412$). La comparaison des relations entre la céruloplasmine sérique des bovins et des moutons révéla la présence d'une différence appréciable entre ces deux espèces. L'activité de la céruloplasmine augmenta plus rapidement chez les moutons que chez les bovins, pour les diverses concentrations de cuivre analysées à cette fin ($P < 0,0001$). L'intercept de la relation de régression ovine se révéla aussi plus bas ($P < 0,0001$). Les auteurs notèrent un coefficient de corrélation de 0,35 entre l'activité de la céruloplasmine sérique et les concentrations hépatiques de cuivre, chez les bovins, indice que la relation mathématique n'était pas bien définie. L'activité de la céruloplasmine sembla en corrélation plus étroite avec les concentrations sériques ou plasmatiques de cuivre, comme le révéla la

comparaison avec les concentrations correspondantes du foie en cuivre.

Mots clés: déficience en cuivre, céruloplasmine, metalloenzyme, activité oxydasique, bovins, moutons.

INTRODUCTION

Ceruloplasmin is a metalloenzyme with oxidase activity that is associated with iron and copper homeostasis (1,2). Each molecule of ceruloplasmin contains six to eight atoms of copper which influence its biological activity (1). Ceruloplasmins have been isolated from several animal species including man and have a similar chemical structure (3,4). Biochemical properties of ceruloplasmin such as optimal pH are often similar in various animal species (5).

In man, ceruloplasmin has been utilized diagnostically for a variety of clinical and pathological conditions including rheumatoid arthritis, liver disease, chronic infections, genetic diseases such as Wilson's disease, copper deficiency and malignancies such as Hodgkin's disease (1,2). In domestic animals, ceruloplasmin has been used diagnostically to a limited extent to investigate copper deficiency (6,7). Copper-deficient animals treated with injectable copper preparations show increased ceruloplasmin activity and blood copper concentrations (6,8).

The purpose of the present study was to investigate the relationship between ceruloplasmin activity and copper concentrations in the serum of sheep and cattle with a normal or deficient copper status. In cattle, the relationship between ceruloplasmin activity and hepatic copper content and the

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differences between serum and plasma ceruloplasmin activity were also investigated.

MATERIALS AND METHODS

SAMPLE COLLECTION

Blood samples were collected from cattle and sheep from various locations in Saskatchewan. Clotted and in certain instances, heparinized blood was obtained to evaluate serum or plasma copper concentrations and ceruloplasmin oxidase activity. Liver tissue was collected for copper determination using needle biopsy techniques in selected cattle herds or from animals which had been bled prior to slaughter.

TISSUE ANALYSIS

Serum or plasma copper concentrations were determined using standard atomic absorption spectrophotometric techniques (9). Blood samples were diluted 1:1 with 1.4 M hydrochloric acid and deproteinated with 1.23 M trichloroacetic acid. After the mixture was filtered and centrifuged, the supernatant was analyzed for copper using a Unicam SP 1900 atomic absorption spectrophotometer at a wavelength of 324.8 μm . Following acid digestion using a nitric:sulfuric: perchloric acid mixture, liver copper concentrations were determined in a similar manner (10).

ENZYME ANALYSIS

The oxidase activity of ceruloplasmin in plasma or serum was determined using a colorimetric enzyme assay (11). The oxidation of *p*-phenylenediamine dihydrochloride by ceruloplasmin was measured in samples incubated for one hour at 37°C at pH 6.0. Following the incubation period, the enzyme reaction was stopped by the addition of sodium azide. The enzyme activity was determined by measuring the absorbance of the oxidation product with a Beckman DU 8 spectrophotometer at 530 m μ . Based on physical and biochemical data (12,13), the oxidase activity indicated by the absorbance was converted to the ceruloplasmin concentration expressed in mg/L.

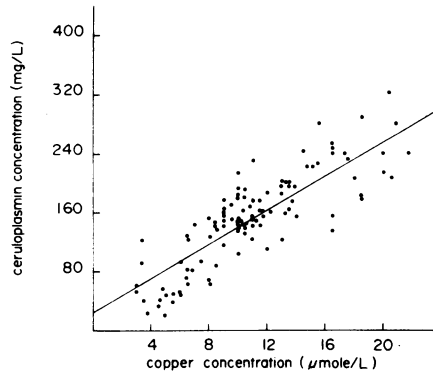


Fig. 1. The relationship between serum ceruloplasmin concentration and serum copper concentrations in cattle ($n = 116$). The linear regression equation describing ceruloplasmin activity is: $y, \text{mg/L} = 11.6 [\text{copper concentration } (x, \mu\text{moles/L})] + 24.8$. The correlation coefficient (r) is 0.83.

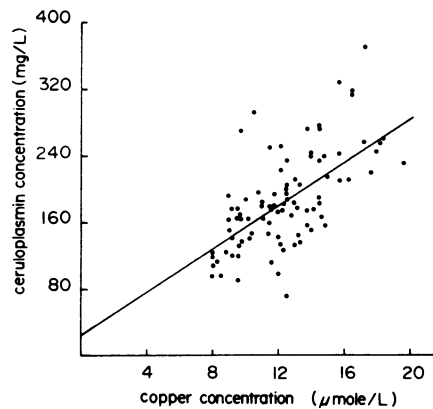


Fig. 2. The relationship between plasma ceruloplasmin concentration and plasma copper concentrations in cattle ($n = 87$). The linear regression equation describing ceruloplasmin activity is: $y, \text{mg/L} = 12.9 [\text{copper concentration } (x, \mu\text{moles/L})] + 25.3$. The correlation coefficient (r) is 0.60.

DATA ANALYSIS

Ceruloplasmin levels and copper concentrations in the plasma, serum or liver were described statistically using linear regression analysis. Statistical comparisons of the slopes and intercepts (14) of the appropriate linear regression equations were used to identify differences in ceruloplasmin activity between cattle serum and plasma samples or cattle and sheep serum samples.

RESULTS

The relationship between ceruloplasmin activity and copper concentrations in serum from 116 cattle is

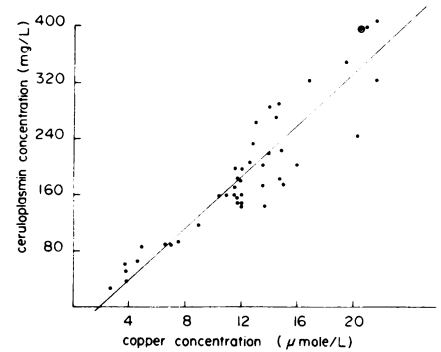


Fig. 3. The relationship between serum ceruloplasmin concentration and serum copper concentrations in the sheep ($n = 45$). The linear regression equation describing ceruloplasmin activity is: $y, \text{mg/L} = 18.3 [\text{copper concentration } (x, \mu\text{moles/L})] - 32.8$. The correlation coefficient (r) is 0.92.

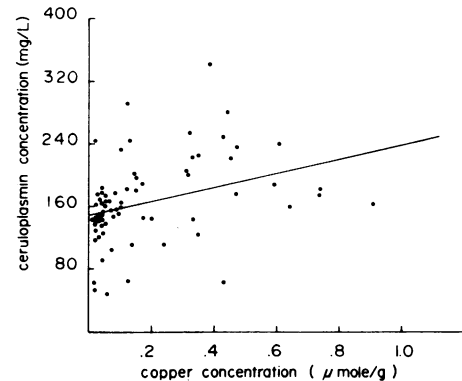


Fig. 4. The relationship between serum ceruloplasmin concentration and hepatic copper concentrations in cattle ($n = 72$). The linear regression equation describing ceruloplasmin activity is: $y, \text{mg/L} = 90.2 [\text{copper concentration } (x, \mu\text{moles/g})] + 150.0$. The correlation coefficient (r) is 0.35.

illustrated in Figure 1. A correlation coefficient of 0.83 was obtained, relating ceruloplasmin activity and serum copper concentrations. In Figure 2, similar comparisons relating ceruloplasmin activity with plasma copper concentrations from 87 cattle are shown. A correlation coefficient of 0.60 was determined in this comparison. In serum copper samples from 45 sheep, a comparable relationship with a correlation coefficient of 0.92 was seen (Fig. 3). In each instance, the relationship between ceruloplasmin and copper remains linear throughout the entire spectrum of copper values which were investigated, including the high normal range of values [normal

TABLE I. The Relationship Between Ceruloplasmin Activity^a and Blood Copper Concentrations^b Using Linear Regression Analysis

Sample	N ^c	Slope (m)	Intercept (b)	Correlation Coefficient (r)
Bovine plasma	87	12.9 ^d	25.3 ^c	0.60
Bovine serum	116	11.6 ^d	24.8 ^{ef}	0.83
Ovine serum	45	18.3 ^d	-32.8 ^f	0.92

^aCeruloplasmin activity — mg/L

^bCopper concentration — μ moles/L

^cNumber of samples

^dAll slopes different from each other, $P < 0.0001$

^eIntercepts — bovine serum vs bovine plasma, $P = 0.412$

^fIntercepts — ovine serum vs bovine serum, $P < 0.0001$

range $> 11 \mu$ mole copper/L (8)].

Table I summarizes the relationships between ceruloplasmin activity and blood copper concentrations in cattle and sheep obtained using linear regression analysis. Comparisons between bovine serum and plasma relationships indicate that ceruloplasmin activity at corresponding copper concentrations is lower in serum samples. The slopes of the linear regression relationships are greater in the plasma study as compared to the serum study, though no difference was observed in the corresponding intercepts. Comparisons between the ovine and bovine serum relationships indicate that significant differences are present with respect to both slope and intercept values.

In 72 cattle, correlations between hepatic copper concentrations and serum ceruloplasmin activity (Fig. 4) illustrate that the relationship is not as well defined as compared to the plasma or serum studies. The correlation coefficient is 0.35, with the linear regression line significantly different from zero ($P = 0.0012$). The equation for the regression line is $Y = 90.2(X) + 150.0$ where ceruloplasmin activity is measured in mg/L and hepatic copper concentration is measured in μ mole/g.

DISCUSSION

Ceruloplasmin appears to be a useful indicator of nutritional copper status in cattle and sheep. Ceruloplasmin activity and the serum or plasma copper concentration decreases with nutritional copper depletion of ruminants. However, McMurray (16) has

reviewed this subject and indicates that a number of variables must be considered to fully evaluate the copper status. Similar correlations between ceruloplasmin and blood copper concentrations have been reported by other investigators. In cattle, correlation coefficients as high as 0.93 have been observed using plasma samples (8). In sheep, comparisons between serum ceruloplasmin and whole blood copper concentrations have also produced good correlations ($R = 0.75$) (15). It is not surprising that good correlations are observed, since ceruloplasmin is reported to contain greater than 95% of the circulating copper in normal animals (2).

Since ceruloplasmin synthesis occurs in the liver, a major site of copper storage, it could be expected that ceruloplasmin activity would be a useful indicator of hepatic copper concentrations. The present study in cattle and another study in sheep (15) demonstrate that the correlations are poor. In the ovine study, correlation coefficients relating plasma or serum ceruloplasmin activity and hepatic copper concentrations were 0.46 and 0.48 respectively. It appears that ceruloplasmin activity is poorly related to hepatic copper concentrations. The activity is probably maintained at the expense of hepatic copper stores and would not necessarily reflect these reserves until the stores have been depleted. Under conditions associated with a normal copper status, most of the copper which is present in the liver is probably sequestered in a storage pool and is unlikely to correlate closely with the functional copper pool that is associated with ceruloplasmin. Other investigators (7,8) have observed a reduction in ceruloplasmin activity in

copper-deficient cattle, though no correlation coefficients with hepatic copper concentrations were stated.

From a diagnostic perspective, ceruloplasmin may have applications in many animal species. Ceruloplasmin has been isolated from most animal species and has a similar structure with similar biochemical properties (3,5). Differences between cattle and sheep ceruloplasmin were identified in the present study. Biological or environmental variables such as age (6) or molybdenum status (17) may not influence ceruloplasmin activity directly though these variables would potentially alter serum or plasma concentrations and affect the diagnostic interpretation of copper status. In contrast, copper deficient animals which have been supplemented with copper do have elevated ceruloplasmin levels (6,8) which can allow for a simple clinical evaluation of a biological treatment response.

From a practical point of view, ceruloplasmin is very stable. Frozen ceruloplasmin will retain its activity for several weeks (6,18). Loss of activity during shipping or handling does not pose a major problem. The linear response which was observed throughout the entire range of copper levels suggests that ceruloplasmin is a reliable indicator of copper status in both the deficient and high normal ranges. The activity did not plateau in the normal range which may have been anticipated for a nutritionally-dependent response. Those responses which plateau in the normal range have limited clinical or diagnostic usefulness. Since ceruloplasmin activity does vary with sample forms, such as serum versus plasma (16,17) or species differences, different laboratory standards must be utilized. McMurray (16) suggested that ceruloplasmin is sequestered during blood clot formation, resulting in relatively lower serum activity. The present study (Table I) also demonstrates that the relationships between plasma or serum copper concentrations and ceruloplasmin activity are not identical. Plasma has more ceruloplasmin activity per μ mole/L of copper than serum, suggesting a relatively greater sequestration of ceruloplasmin than copper during the clotting process.

Under routine diagnostic labora-

tory conditions, ceruloplasmin appears to be a useful diagnostic aid to evaluate copper status in cattle and sheep.

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