Influence of Age and Sex on Serum Copper and Ceruloplasmin Levels

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Serum copper and ceruloplasmin concentrations were determined in 180 normal male subjects and 44 female subjects, age 20 to 89 years. A small but significant increase (p < .025) in serum copper concentrations was observed with age in the male population; none was observed in the females. Serum ceruloplasmin concentrations failed to change with age in either group. In contrast with most previous studies, no difference in serum copper levels existed between the male and female populations. Serum ceruloplasmin levels, however, were significantly higher in the females.

The range of serum copper concentrations in normal subjects has been well defined (Cartwright, Markowitz, Shields, & Wintrobe, 1960; Caruthers, Hobbs, & Warren, 1966; Halsted, Hackley, & Smith, 1968; Henkin, Schuman, Schuman, & Bronzert, 1973; Herring, Leavell, Paixao, & Yoe, 1960; Schenker, Hellerstein, Jungrig, & Polishuk, 1971; Sinha & Gabrieli, 1970; Sunderman & Roszel, 1967; Zachheim & Wolf, 1972). Higher plasma copper concentrations generally have been found in females than in males (Cartwright et al., 1960; Herring et al., 1960; Rice, 1962; Sinha & Gabrieli, 1970; Zachheim & Wolf, 1972). Two studies have examined the effect of age on serum copper concentrations and both have reported significant increases with age in male populations (Harman, 1965; Herring et al., 1960).

The range of serum ceruloplasmin concentrations in normal subjects also has been well defined using a variety of analytical techniques (Caruthers et al., 1966; Cox, 1966; Haralambie, 1969; Laurell, Kullander, & Thorell, 1969; Morell, Windsor, Steinleib, & Scheinberg, 1968; Ravin, 1961; Sunderman & Nomoto, 1970). Females again appear to have higher concentrations than males (Cartwright et al., 1960; Cox, 1966). Although changes in serum ceruloplasmin levels with age have been reported through childhood and adolescence (Cox, 1966), no information was uncovered which described an effect of age on serum ceruloplasmin levels in adults.

The present study was undertaken to determine, by reviewing previous publications and by adding the results of our own experiences, whether or not there are age or sex differences in serum copper or ceruloplasmin levels in an adult population.

Methods

Subjects.—Single serum samples for determination of copper and ceruloplasmin concentrations were obtained from 180 male subjects and 44 female subjects at the Oklahoma State Penitentiary and from the domiciliary care veterans' facility at Central State Hospital. Individuals with acute intercurrent illnesses, hepatic, renal or symptomatic cardiovascular disease were eliminated from the study. Some of the older patients did have evidence of atherosclerosis, compensated heart failure or mild diabetes mellitus.

Techniques.—Disposable plastic syringes and collection tubes were used throughout the study to minimize contamination. After the blood was centrifuged, serum samples were re-
frigerated until copper and ceruloplasmin analyses were completed.

Copper analyses were performed with a Perkin-Elmer Model 303 atomic absorption spectrophotometer using techniques previously described modified only by using 7.5% trichloracetic acid as a protein precipitant rather than 3N HCl (Lindeman, Clark, & Colmore, 1971). With this method, the analytical sensitivity for the copper determination was 0.2 \( \mu g/ml \) for percent absorption and a relative detection limit of 0.005 \( \mu g/ml \). Recovery of added copper using techniques similar to those used for zinc ranged between 96 and 104%.

Plasma ceruloplasmins were assayed using commercially available immunodiffusion plates (Hyland, Inc., normal range 20-35 mg/100 ml.). Some variance in the mean serum ceruloplasmin concentrations was encountered in the five lots of immunodiffusion plates utilized in this study. For this reason, the results from the five different lots are separately identified in Figure 2. All female samples were studied with two lots.

Statistical methods.—The mean, standard deviations, correlation coefficients, and regression equations were calculated using standard techniques. Analysis of variance was utilized to test the hypothesis that the slopes of the age regression lines for serum copper and ceruloplasmin and the ceruloplasmin vs. copper comparison were equal to zero for males and females. Analysis of covariance was utilized to test the hypothesis that the serum copper and ceruloplasmin concentrations for males were equal to those of females.

Results

The mean ages, serum copper, and serum ceruloplasmin concentrations ± standard deviations for 180 male and 44 female subjects are shown in Table 1. The correlation coefficients and regression equations for serum copper concentrations vs. age, serum ceruloplasmin concentrations vs. age, and serum ceruloplasmin vs. serum copper also are shown in Table 1. When tests of homogeneity for the regression slopes comparing the male and female populations were applied, no significant differences were noted comparing serum copper vs. age or serum ceruloplasmin vs. age.

The correlations between serum copper concentrations and age in male and female subjects are shown in Figure 1. A significant increase (\( p < .025 \)) in serum copper levels with age was observed in males; no significant change with age was observed in females. When corrected for age differences, the females had higher serum copper levels than males, but this difference was not statistically significant.

The correlations between serum ceruloplasmin concentrations and age in male and female subjects are shown in Figure 2. No significant change in serum ceruloplasmin concentrations with age was observed in either the male or female subjects. Serum ceruloplasmin levels were significantly higher in females than in males.

Highly significant correlations (\( p < .005 \)) existed between the serum ceruloplasmin and copper concentrations in both male and female subjects. The correlation coefficient in males was .36: in females, it was .84. When the correlation coefficients for the samples using the different lots of immunodiffusion plates were calculated separately, the individual correlation coefficients in males ranged in the five lots from .30 to .88 with a mean of .53.

Discussion

Copper is an essential component of many enzymes present in biological systems, the most important being cytochrome oxidase. Approximately 95% of plasma copper is incorporated into ceruloplasmin where it is not exchangeable with ionic copper and is released only when the protein molecule is catabolized. The remainder is bound loosely to albumin (Gubler, Lahey, Cartwright, & Wintrobe, 1953) and amino acids (Neumann & Sass-Kortsak, 1967)
Fig. 1. Serum copper concentrations in 180 males and 44 females vs. age. The solid lines represent the calculated regression equations.

Fig. 2. Serum ceruloplasmin concentrations in 180 males and 44 females vs. age. The results from each of the 5 lots of immunodiffusion plates utilized are shown by different symbols.
where it can be exchanged readily with tissue and enzyme copper. Specific copper binding proteins also exist within the red cell (erythro- cuprein), brain (cerebrocuprein), and liver (hepatocuprein).

Table 2 lists selected studies showing the normal ranges of serum copper concentrations in males and females using both spectrophotometric and atomic absorption techniques. Our values ± S. D. agree closely with these prior studies.

Table 3 lists selected studies showing normal ranges of serum ceruloplasmin concentrations in males and females using several techniques. The p-phenylenediamine (PPD) oxidase method had been used most frequently in males and females using several techniques. The other methods (Sunderman & Nomoto, 1970). Although the immunodiffusion technique is commercially available and widely used because of the ease of the determination, little information is available correlating immunodiffusion results with other techniques (Rosenberg, Strickland, Feng, & Blackwell, 1971).

Higher serum copper and ceruloplasmin concentrations have been reported in females than in males (Cartwright et al., 1960; Cox, 1966; Herring et al., 1960; Rice, 1962; Sinha & Gabrieli, 1970; Zachheim & Wolf, 1972). Both serum copper and ceruloplasmin are increased in pregnancy and after estrogenic oral contraceptives (Caruthers et al., 1966; Hasted et al., 1968; Laurell et al., 1969; Schenker et al., 1971). In previous studies, the female populations have not been screened to eliminate subjects who were pregnant or on oral contraceptives. None of the subjects in this study was pregnant and none was taking oral contraceptives. This may account for our failure to find a significant difference in serum copper concentrations between male and female.

Two studies in all male populations have reported a significant increase in serum copper concentrations with age (Harman, 1965; Herring et al., 1960). Herring et al. (1960) only surveyed persons from age 10 to 50 years with very few over age 40 years. Harman (1964) shows a linear increase in serum copper concentrations with age with a mean value of 124 mg/100 ml. at age 20 and 145 mg/100 ml. at age 60 years. The same author noted that copper is an excellent catalyst and speculated that when it is present in increased amounts it might increase the rate of tissue peroxidation and hence accelerate atherosclerosis. He also noted that patients with a past history of acute myocardial infarction had significantly higher serum copper levels than a matched group without infarction.

Serum copper concentrations are reported to be increased in a number of chronic illnesses such as coronary atherosclerosis with or without infarction and/or heart failure, cere-
bral atherosclerosis, essential hypertension, diabetes mellitus, chronic pulmonary disease, and in various hematologic disorders (Herring et al., 1960; Sinha & Gabrieli, 1970). Although patients with acute illnesses were excluded from this study, it would be difficult to eliminate chronic illnesses in an aged population and no attempt was made to do so.

Summary
Serum copper and ceruloplasmin concentrations were determined in 180 male subjects and 44 female subjects, age 20 to 89 years. A small but significant increase ($p < .025$) in serum copper concentrations was observed with age in the male population; none was observed in the females. Serum ceruloplasmin concentrations failed to change in either group with age. In contrast to most previous studies, we found no difference in serum copper levels in the male and female populations; there was, as previously reported, a significant increase in the serum ceruloplasmin levels in the females. The significance of the increase in serum copper concentrations in the male population with age remains undefined but it might conceivably play a role in the aging processes.

References


