

## Serum Copper Levels in Normal Sri Lankan Subjects and in Various Pathological States

by

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**SUMMARY.** The levels of serum copper were determined by atomic absorption spectrophotometry in normal subjects, pregnant women, and in patients with infectious hepatitis, nephrotic syndrome and cirrhosis. The normal range was  $11$  to  $44.54 \mu \text{mol l}^{-1}$  with a mean  $\pm \text{SD}$  (standard deviation) of  $23.9 \pm 9.1 \mu \text{mol l}^{-1}$ . Lower levels were seen in cirrhosis, Wilson's disease and the nephrotic syndrome and higher levels were seen in pregnant women and in patients with hepatitis.

### INTRODUCTION

It has been established that copper in the living organism is concerned with catalytic functions.<sup>1</sup> Copper was recognised to form an essential constituent of a number of enzymes. Trace amounts of copper are essential for life but excess of copper is toxic.

Plasma copper can be subdivided into two fractions in which a greater amount of it is bound to the  $\alpha_2$ -globulin and known as caeruloplasmin. A small fraction of plasma copper (2%) is bound to serum albumin and is referred to as the non-caeruloplasmin bound copper<sup>2</sup>, or the "Direct Reacting" copper fraction since it directly reacts with diethyl dithio carbamate<sup>4</sup>. Caeruloplasmin-copper is known as the "Indirect Reacting" fraction of plasma copper. There is another fraction of serum copper bound to amino acids. This fraction is extremely small and it may have an important function in the transport of copper across the cell membranes.<sup>7</sup> Caeruloplasmin deficiency results in an increase of the ionic copper in plasma. The ionic copper which cannot form a complex with the albumin is deposited in tissues of the body and the balance excreted in the urine chelated with amino acids<sup>3</sup>. This occurs in Wilson's disease.

Normal values for caeruloplasmin have been determined<sup>5</sup> by a modified enzymatic method<sup>6</sup>. The present study investigated the total serum copper levels in normal Sri Lankan subjects, in several pathological states and in pregnancy.

## MATERIALS AND METHODS

## Reagents and equipment

Hydrochloric (HCl), perchloric ( $\text{HClO}_4$ ) and nitric ( $\text{HNO}_3$ ) acids used in the wet digestion of the serum were of analytical grade. Analysis of copper was carried out using a Varian AA5 model atomic absorption spectrophotometer.

## Blood Samples

## 1. Normal

For the establishment of the clinical norm for serum copper, blood samples were obtained from the Blood Bank, General Hospital, Colombo. Glass containers used for collection were washed with dilute HCL(2N) and rinsed with glass-distilled water. Screening of these blood samples for haemoglobin, Wasserman reaction, total proteins and malarial parasites were carried out by the Blood Bank as routine procedures.

## 2. Pathological

Samples of blood received by the Department of Biochemistry, Medical Research Institute, Colombo, from various hospitals in the island for routine assays for hepatitis cirrhosis, Wilson's disease and other abnormalities were used to estimate the serum copper levels.

## 3. Pregnancy

Samples of blood from women in different stages of pregnancy were obtained from the Castle Street Hospital for Women, Colombo. For this collection containers used were washed with dilute HCL (2N) and glass - distilled water.

## Assay of Serum Copper

Serum (0.5 - 1.5ml) diluted ten times with distilled water was wet-digested with  $\text{HClO}_4$  (1.0ml of 70% v/v) and  $\text{HNO}_3$  (5ml of 70% v/v). The digest was evaporated to expel the remaining  $\text{HClO}_4$  and cooled. To the remaining solutions HCl, (0.5ml of 36% v/v) was added followed by distilled water to make up the volume to 10ml. This solution was analysed using Varian AA5 atomic absorption spectrophotometer.

## RESULTS AND DISCUSSION

## Normal Subjects

Fig. 1 gives the frequency distribution of total serum copper in normal Sri Lankan subjects. Out of a total of 65 observations, normal range was found to be 11-44.5  $\mu\text{mol l}^{-1}$  with a mean value of 23.87  $\mu\text{mol l}^{-1}$  (s.d. 9.00).

Histogram of Total Copper

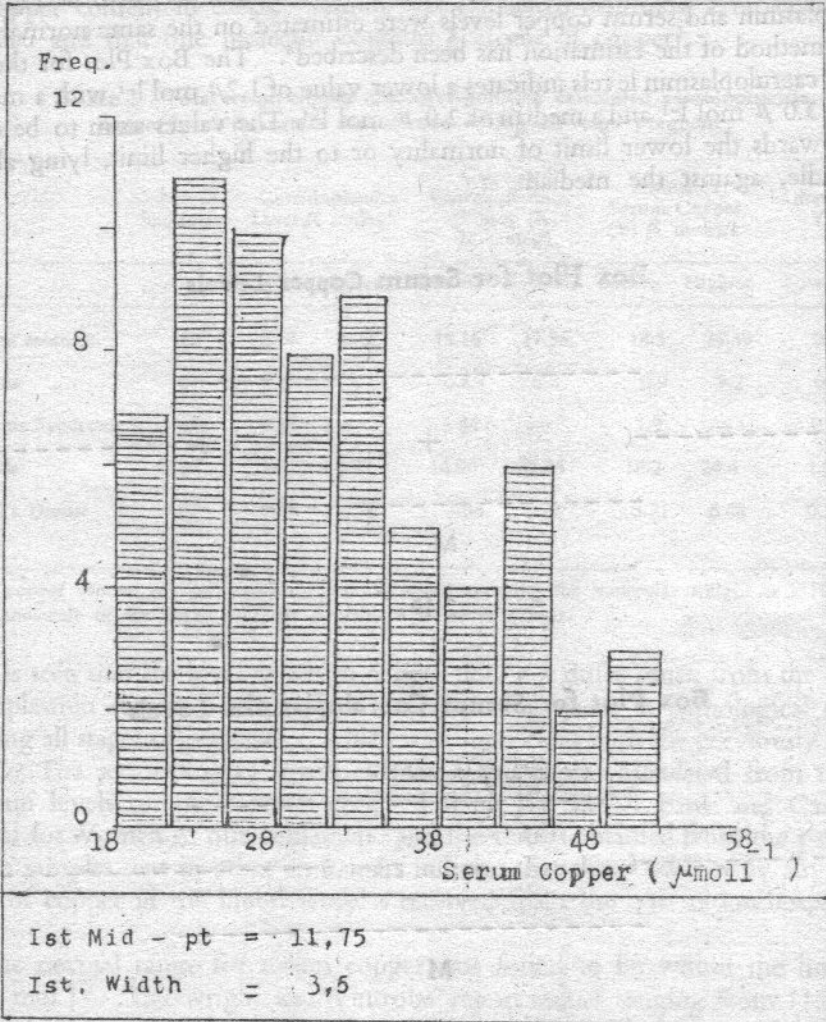
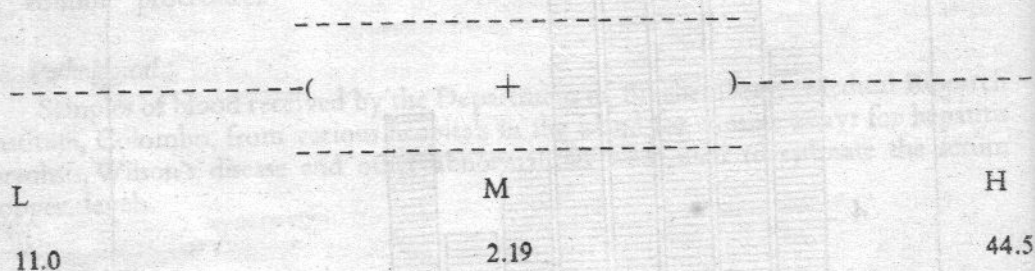


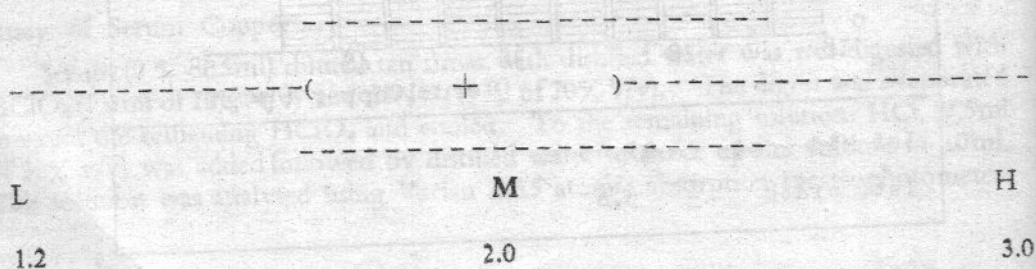
Fig. 1. Histogram of total serum copper.

Fig. 2 gives the 'Box Plot' diagram of the serum copper and caeruloplasmin levels in normal Sri Lankan subjects. It is seen from the Box Plot for serum copper, that the lower limit is  $11.0 \mu \text{ mol l}^{-1}$  with a maximum of  $44.5 \mu \text{ mol l}^{-1}$  and a median of  $21.9 \mu \text{ mol l}^{-1}$ . The plot indicates that the values are distributed more towards the lower limit rather than to the higher. The distribution seems to be log normal. Caeruloplasmin and serum copper levels were estimated on the same normal samples and the method of the estimation has been described<sup>8</sup>. The Box Plot for the corresponding caeruloplasmin levels indicates a lower value of  $1.2 \mu \text{ mol l}^{-1}$  with a maximum value of  $3.0 \mu \text{ mol l}^{-1}$  and a median of  $2.0 \mu \text{ mol l}^{-1}$ . The values seem to be unbiased either towards the lower limit of normality or to the higher limit, lying almost in the middle, against the median.

### Box Plot for Serum Copper Levels



### Box Plot for Serum Caeruloplasmin Levels



L = Lowest

M = Median

H = Highest

Fig. 2. Box Plots for serum copper and caeruloplasmin levels.

## Other Subjects

Table 1 gives the total serum copper and the corresponding calculated caeruloplasmin copper levels in certain pathological states and during all stages of pregnancy. The copper content in caeruloplasmin was calculated assuming a molecular weight as 151000 and that one molecule contains 8 atoms of copper<sup>5</sup>.

TABLE 1. Total serum copper and corresponding calculated caeruloplasmin copper levels in certain diseases and during all stages pregnancy

| Case               | No. of Specimens | Caeruloplasmin Level $\mu$ moles/L |         | Calculated Caeruloplasmin Copper (X) $\mu$ moles/L |         | Estimated Serum Copper (Y) $\mu$ moles/L |         | Difference between Y and X |         |
|--------------------|------------------|------------------------------------|---------|--|---------|--|---------|----------------------------|---------|
|                    |                  | Lowest                             | Highest | Lowest   | Highest | Lowest                                   | Highest | Lowest                     | Highest |
| Pregnant mothers   | 12               | 2.27                               | 3.42    | 18.16  | 27.36   | 18.3                                     | 28.39   | 0.14                       | 0.03    |
| Cirrhosis          | 07               | 0.1                                | 1.1     | 0.8  | 8.8     | 0.9                                      | 9.2     | 0.1                        | 0.4     |
| Nephrotic Syndrome | 01               | 0.48                               | —       | 3.84   | —       | 3.9                                      | —       | 0.06                       | —       |
| Hepatitis          | 04               | 1.87                               | 2.77    | 14.96  | 22.26   | 16.2                                     | 24.4    | 1.24                       | 2.14    |
| Wilson's Disease   | 02               | 0.38                               | 0.78    | 3.04   | 6.24    | 3.21                                     | 6.68    | 0.17                       | 0.44    |

The copper content of caeruloplasmin was calculated assuming the molecular weight as 151000 and that one molecule of the enzyme protein contains 8 atoms of copper.

It is seen that the level of serum copper does not differ much from the calculated caeruloplasmin copper levels both in the normal subjects, in pathological specimens or during all stages of pregnancy, which is in agreement with the previously published results<sup>7</sup>. The serum copper levels estimated and those calculated from the caeruloplasmin levels on the samples received from the Blood Bank and Castle Street Hospital for Women in our containers, and the results obtained from the pathological random samples sent in other containers indicate that there was hardly any contamination of copper in the blood samples received from the out-station hospitals.

The normal range for serum copper was found to be within the limits 11 to 44.5  $\mu$  mol l<sup>-1</sup>. Cartwright and Wintrobe<sup>2</sup> report values ranging from 11.8 to 24.9  $\mu$  mol l<sup>-1</sup>. Our values are higher than the reported values which could be attributed to the sensitivity of the atomic absorption spectrometric assay. This method estimates the copper attached to the albumin, to  $\alpha_2$ -globulin which is caeruloplasmin and serum copper bound to amino acids. In Wilson's disease estimated copper content and the calculated copper content of the caeruloplasmin are much reduced and ranged from 3.21 to 6.68  $\mu$  mol l<sup>-1</sup> and 3.04 to 6.24  $\mu$  mol l<sup>-1</sup>, respectively. The differences between them were 0.17 to 0.44 mol l<sup>-1</sup>.

During pregnancy there is hypercupraemia in the region of  $28 \mu \text{ mol l}^{-1}$  the copper being contributed by the caeruloplasmin which is elevated in pregnancy. However, in cirrhosis, the highest value obtained was less than  $10 \mu \text{ mol l}^{-1}$ . A similar pattern is seen in the nephrotic syndrome. In cirrhosis the diseased liver cells release the caeruloplasmin - copper, giving rise to ionised copper which is excreted in the urine.

The estimated serum copper was found to differ very marginally in the case of cirrhosis, nephrotic syndrome, Wilson's disease, in the range 0.1 - 0.4, 0.06, 0.17-0.44  $\mu \text{ mol l}^{-1}$ , respectively, from the calculated caeruloplasmin copper. However in the case of hepatitis the lowest value for the difference between the estimated serum copper and that of copper calculated from the caeruloplasmin content is 0.1% and the highest value is 11.1%, most of the values being greater than 5. Therefore estimation of the copper from the caeruloplasmin content gives an approximate index of the serum copper. For clinical purposes this may be sufficient when considering the labour involved in the determination of the serum copper and the need for an atomic absorption spectrophotometer.

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#### REFERENCES

1. CANTAROW, A and TRUMPER M (1962). *Clinical Biochemistry* 6th ed. p. 262. Philadelphia, London: W.B. Saunders Company.
2. CARTWRIGHT, G. E. and WINTROBE, M. M. (1964). Copper metabolism in normal subjects, *American Journal of Clinical Nutrition* 14, 224-237.
3. DAVID, Y. H., (1960) In *Inborn Errors of Metabolism* p. 78-74. Chicago, U.S.A.: The Year Book Publishers.
4. GUBLER, L. J., BROWN H, CARTWRIGHT, G. L. and WINTROBE M. M. (1957). Studies on Copper metabolism. XXII. Portal (Laenec's) cirrhosis of liver. *Journal of Clinical Investigation* 36, 1208-121.
5. HOLMBERG, C. G., and LAURELL, C. B. (1948). Investigations in Serum Copper. II. Isolation of the copper-containing protein and a description of some its properties. *Acta Chemica Scandinavica* 2, 550-53.
6. KING, J. (1965). In *Practical Clinical Enzymology*, pp. 112-113. London: D VanNostrand Company Ltd
7. SASS-KÖRTASK, A. (1965). Copper metabolism, *Advances in Clinical Chemistry* 8, 1-67.
8. SENTHESHANMUGANATHAN, S. DHARMADASA, D. WIJERATNE, S. and TAMMANPILA, N. J. (1968), A modified method for the estimation of caeruloplasmin levels in human serum, the establishment of the clinical norm and studies of the caeruloplasmin levels in various pathological states. *Journal of the National Science Council of Sri Lanka*, in print.