SERUM COPPER AND ITS RELATIONSHIP TO SERUM IRON IN PATIENTS WITH NEOPLASTIC DISEASE

BY

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The importance of serum copper in the transport of copper throughout the body was established by Sachs, Levine, Schmit, and Hughes (1941) when they observed that the oral administration of varying amounts of copper sulphate was followed by a definite rise in the copper content of the serum. Later Yoshikawa, Hahn, and Bale (1942) obtained a similar result by feeding radio-active copper salts to normal and anaemic dogs. After its ingestion the tagged metal rapidly appeared in the serum, reaching its peak concentration within two to five hours. These observations indicate that the role of serum copper in copper metabolism is analogous to that of serum iron in iron metabolism, and, by permitting valid conclusions to be drawn from estimations of serum copper levels, have facilitated the study of copper metabolism in man.

The purpose of this paper is to present the values of serum copper obtained in normal subjects and in patients with neoplastic disease unaccompanied by haemorrhage, and to compare the iron and copper concentrations in the serum of these patients.

Methods

Serum copper was determined by a minor modification of the technique described by Cartwright, Jones, and Wintrobe (1945). This method consists of the triple extraction of the trichloroacetic acid precipitate of serum and the development of the coloured copper-dithiocarbamate complex, the resultant colour being measured directly in the aqueous extract. Difficulty was encountered in obtaining optically clear aqueous extracts for photometry, however, and extraction of the coloured complex with amyl alcohol, as originally suggested by McFarlane (1932), was therefore employed. The procedure finally adopted was as follows. The aqueous phase, obtained by following the procedure of Cartwright *et al.*, was made up to a volume of 20 ml. with distilled water in a graduated test-tube, 10 ml. of amyl alcohol added, and the test tube shaken vigorously for two minutes. The amyl alcohol phase was then separated by centrifugalization and transferred by a Pasteur pipette to a thoroughly dried 1 cm. optical cell. Colour was measured in a Spekker absorptiometer using the Ilford spectrum filter, No. 601. Clear extracts of the coloured copper complex were consistently obtained when this procedure was followed.

Serum iron was estimated by the method of Fitzpatrick and Howells (1949), by whom it has already been described in detail. The Haldane method was used for haemoglobin determination.

Results

Copper was estimated in triplicate in five different sera (Table I) and the results showed good agreement, the maximum difference being 4.5%.

TABLE I
TRIPPLICATE ESTIMATIONS OF SERUM COPPER

Serum	Copper Content ($\mu\text{g. \%}$)		
1	89	93	93
2	151	150	153
3	326	326	324
4	115	112	112
5	110	115	115

In 20 healthy adult males, the mean and its standard error of serum copper values were $105 \pm 5.74 \mu\text{g. \%}$ with a range of 74 to 151 $\mu\text{g. \%}$. The corresponding values in 20 healthy adult females were $120 \pm 6.72 \mu\text{g. \%}$ and 84 to 168 $\mu\text{g. \%}$. This difference between the sexes was not significant. These results compare favourably with those already reported (Table II); only in the series of Nielsen (1944a) and Munch-Petersen (1948) is the observed sex difference in serum copper significant.

TABLE II
SUMMARY OF PUBLISHED DATA ON SERUM COPPER IN NORMAL SUBJECTS

Investigator	Serum Copper ($\mu\text{g. \%}$)					
	Males			Females		
	No.	Mean	Range	No.	Mean	Range
Nielsen (1944a)	30	110	75-165	100	123	75-165
Cartwright <i>et al.</i> (1945)	25	116	92-134	25	131	103-159
Munch-Petersen (1948)	50	108	81-164	50	118	73-152
Cartwright <i>et al.</i> (1948)	52	114	86-141	53	123	87-161
Brendstrup (1948)	40	113	84-162	40	120	89-152
Present investigation	20	105	74-151	20	120	84-168

Serum copper and serum iron were determined in 19 patients with malignant disease unaccompanied by haemorrhage, and the results are set out in Table III. The mean value for serum copper in these patients was $223 \pm 14.18 \mu\text{g. \%}$; the difference between serum copper levels in these patients and those of normal subjects was highly significant. The values for serum iron had a mean of $67 \pm 5.96 \mu\text{g. \%}$ and were significantly lower than those obtained in normal subjects by the potassium ferricyanide method (Pirrie, 1952). In Fig. 1 serum copper results are plotted against serum iron results, the latter being grouped in 20 $\mu\text{g. units}$. The relationship between the two is approximately linear, and the individual results for serum copper were found to show a statistically significant inverse correlation to those for serum iron. No significant correlation between serum copper and haemoglobin was established in this series. Thus, of the five patients whose haemoglobin and red cell counts were within normal limits, three had elevated serum copper and lowered serum iron.

TABLE III
SERUM COPPER AND SERUM IRON IN PATIENTS WITH NEOPLASTIC DISEASE UNACCOMPANIED
BY HAEMORRHAGE

Diagnosis	Age (yrs.)	Sex	Hb. (%)	R.B.C. ($\times 10^6$ / c.mm.)	Serum Copper ($\mu\text{g. \%}$)	Serum Iron ($\mu\text{g. \%}$)
Carcinoma of lung	54	M.	114	5.78	184	73
" "	61	M.	64	—	325	40
" "	62	M.	74	4.25	211	96
" "	38	M.	74	3.92	326	65
" "	39	M.	89	—	178	57
" "	43	M.	70	3.85	211	67
" "	67	M.	89	5.01	311	43
" "	40	M.	58	3.65	228	35
" "	63	M.	122	6.51	151	110
Sarcoma of femur	35	M.	70	3.94	262	56
Carcinoma of prostate	69	M.	102	5.49	174	95
" "	71	M.	106	6.12	193	81
" "	58	M.	76	—	138	105
Carcinoma of tongue	67	M.	88	5.02	178	43
Carcinoma of penis	71	M.	105	5.62	124	105
Carcinoma of oesophagus	59	M.	84	4.91	263	30
Carcinoma of breast	67	F.	104	5.84	213	74
" "	58	F.	82	4.45	293	40
" "	38	F.	106	5.37	245	50

FIG. 1.—The relationship between serum copper and serum iron in patients with malignant disease unaccompanied by haemorrhage. Individual results of serum copper (●). Mean serum copper of serum iron groups (x).

Discussion

The effect of various diseases upon serum copper has already been studied. Hypocupraemia is rarely found and is not apparently peculiar to any particular clinical entity. Hypercupraemia occurs in thyrotoxicosis (Nielsen, 1944b), in infectious mononucleosis (Munch-Petersen, 1948), in both acute and chronic infections (Cartwright, Lauritsen, Jones, Merrill, and Wintrobe, 1946), and in pregnancy, in which it appears after the third month and persists until four to seven weeks after parturition (Nielsen, 1944c). In addition, Cartwright, Huguley, Ashenbrucker, Fay, and Wintrobe (1948) have described its occurrence in iron deficiency anaemia, in nephritis with anaemia, and in leukaemia and lymph node disorders.

The significance of hypercupraemia and of the inverse correlation between the degrees of hypercupraemia and hypoferraemia, which were observed in these patients with neoplastic disease, is difficult to assess at present. The assumption that the two metals compete for the same metal-combining protein in the plasma is unjustified in the light of the work of Holmberg and Laurell (1947). The iron-combining protein is a β_1 -globulin, and, although it can combine with copper *in vitro*, (Surgenor, Koechlin, and Strong, 1949) there is no evidence to show that it does so *in vivo*. On the contrary, Holmberg and Laurell (1947) have shown that the main part of native serum copper is found in the α -globulin fraction of the serum. In this connexion, it is interesting to note that there is a conspicuous increase in α_2 -globulins in the serum of patients with neoplastic disease (Shedlovsky and Scudder, 1942; Seibert, Seibert, Atno, and Campbell, 1947).

The occurrence of anaemia in these patients even in the absence of blood loss demonstrates that haemoglobin synthesis is depressed or deranged in the presence of neoplastic disease. A reduced demand for copper, an essential catalyst in haemoglobin synthesis, may be the explanation of the observed hypercupraemia.

Summary

Mean values of 105 ± 5.74 $\mu\text{g. \%}$ and 120 ± 6.72 $\mu\text{g. \%}$ were established for serum copper in 20 healthy adult males and 20 healthy adult females respectively. This sex difference was not significant.

Serum copper levels in 19 patients with neoplastic disease unaccompanied by haemorrhage were significantly higher than normal, and serum iron was significantly lower.

A significant inverse correlation between serum copper and serum iron was observed in these patients.

These findings are briefly discussed.

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