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SERUM COPPER LEVELS IN PREGNANCY AND IN PRE-ECLAMPSIA

BY

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In 1928 Krebs reported that the concentration of copper in the serum was significantly raised in the terminal stages of pregnancy. Since that time this observation has been confirmed by a number of workers (Gorter, Grendel, and Weyers, 1931; Locke, Main, and Rosbash, 1932; Sachs, Levine, and Fabian, 1935; Nielsen, 1944), who have shown that at full term the serum copper level is approximately twice the value found in non-pregnant subjects.

The significance of this raised serum copper level in pregnancy is, however, still obscure. Sachs, Levine, and Fabian (1935) have concluded that it represents a normal, physiological adjustment for the transport of copper from the maternal blood to the foetus, but no evidence is presented in support of this view. Other observations in the literature suggest that the hypercupraemia of pregnancy may, in part at least, be associated with some more specific function. It is well established that certain of the plant phenol oxidases are copper-containing proteins (Kubowitz, 1937, 1938; Keilin and Mann, 1938, 1939). Baker and Nelson (1943) have described a copper protein from kidney which is capable of catalysing the oxidation of adrenaline, and Holmberg in 1944 obtained a blue copper-containing protein from the "euglobulin" fraction of the serum proteins which possessed a laccase-like activity, catalysing the oxidation of *p*-phenylenediamine.

More recently, Holmberg and Laurell (1948) have described a blue protein containing 0.36% Cu derived from the serum globulin fraction which, when added to male plasma to give a final copper concentration of 700 μg./100 ml., increased the histaminolytic activity of the plasma 400-fold. They also point out that the increase in serum copper in pregnancy runs parallel with the increase in the histaminolytic activity of the plasma.

In view of this finding, and since changes in the histaminolytic activity of the plasma have been reported in pre-eclamptic toxaemia (Kapeller-Adler, 1944, 1947), a study of the level of serum copper in this condition has been carried out.

It is known from the work of Abderhalden and Möller (1928) and Boyden and Potter (1937) that the copper in the plasma from non-pregnant subjects exists in a non-dialysable form, and Eisler, Rosdahl, and Theorell (1936), using electrophoretic methods, have stated that the rate of migration of the copper in horse serum is the same as that of the serum albumin. Cohn and Koechlin (1947), on the other hand, have described a copper-combining protein in the β-globulin fraction of human plasma. Experiments have also been carried out therefore to determine the proportion of the copper precipitating with various protein fractions, separated by graded concentrations of sodium sulphate, from the serum of both non-pregnant and pregnant subjects.

Experiments

Materials.—Blood samples have been taken from 42 women with normal pregnancies (37–40 weeks), from 35 subjects four to seven weeks post-partum, from a further nine normal subjects at intervals throughout pregnancy, and from 18 healthy, non-pregnant women in the reproductive age.

Samples have also been obtained from 12 cases admitted to the maternity ward on account of pre-eclamptic toxaemia. The criteria on which this diagnosis was based were a blood pressure of over 160/90 occurring after the thirtieth week of pregnancy in a subject whose blood pressure at the beginning of pregnancy had been within the normal range, together with either albuminuria (ten cases) or oedema (nine cases).

Methods.—The samples were taken into acid-cleaned all-glass syringes, and all glassware used subsequently in the course of the estimations was acid-cleaned and washed with copper-free water distilled over "pyrex" glass.

Copper was estimated in 3 ml. of the separated serum by a modification of the colorimetric method of Eden and Green (1940), based on the reaction of copper with sodium diethyldithiocarbamate. In all experiments in which it was desired to estimate the absolute

amount of copper present, the sample was digested with H_2SO_4, HNO_3, and $HClO_4$; blank estimations were performed on identical amounts of all reagents used.

Results

The values obtained for the serum copper levels of a series of healthy, non-pregnant women, of women late in pregnancy (37–40 weeks), and of women four weeks and five to seven weeks post-partum, together with a small series from cases of pre-eclamptic toxaemia, are shown in Table I.

TABLE I

SERUM COPPER LEVELS (μG.CU/100 ML. SERUM) IN NON-PREGNANT SUBJECTS, IN NORMAL PREGNANCY, AND IN PRE-ECLAMPSIA

Subjects	Mean	Standard Error of the Mean	Range	No. of Cases
Normal				
Non-pregnant ..	106	4.2	78–135	18
Normal pregnancy (37-40 weeks)	230	5.7	180–288*	42
4 weeks post-partum	143	4.5	102–180	24
5–7 weeks post-partum	114	5.4	88–139	11
Pre-eclamptic ..	277†	8.1	235–337	12

* One value of 387 was obtained. Although this has been included in calculating the mean, it has not been included in the range since it differs from the mean by more than three times the standard deviation of the mean.

† Individual values in pre-eclamptics were 250, 312, 255, 337, 275, 277, 235, 281, 252, 283, 285, and 280 μg./100 ml.

It will be seen that in each case the serum copper level in the terminal stages of normal pregnancy is very considerably raised.

A further nine cases attending the ante-natal clinic were followed through pregnancy at approximately three-monthly intervals, and a further estimation was made on each when the patient returned to hospital for the final post-natal examination. Since these subjects were attending the clinics as out-patients it was not possible in each case to obtain blood samples at exactly similar intervals, and the estimations were carried out at times varying from 10 to 16 weeks, 26 to 32 weeks, and 37 to 40 weeks of pregnancy in each case, and again at 6 to 11 weeks post-natally. The results of these estimations are given in Table II.

The findings of these nine cases confirm the earlier observations of Nielsen (1944) that the rise in serum copper occurs at an early stage in pregnancy; it will be seen that the level is significantly increased by the tenth to sixteenth week, although a further slight increase appears to take place until full term; 6 to 11 weeks after delivery the serum copper level appears to have returned once more to the normal level for the non-pregnant subject.

TABLE II

SERUM COPPER LEVELS (μG.CU/100 ML. SERUM) THROUGHOUT NORMAL PREGNANCY AND POST-NATALLY

Case	10–16 Weeks	26–32 Weeks	37–40 Weeks	Post-natal (6–11 Weeks)
1	267	298	288	185
2	182	—	287	129
3	193	270	228	89
4	152	178	(387)*	133
5	182	228	212	126
6	148	190	240	117
7	166	213	237	95
8	183	225	240	110
9	179	165	215	92
Mean	184	221	243	119

* Not included in the mean given in this table since it differs from the mean given in Table I by more than three times the standard deviation of the mean.

The values obtained in pre-eclampsia are also high, the mean for the 12 cases studied being 277 μg./100 ml. (standard error = 8.1) compared with a mean of 230 μg./100 ml. (s.e. = 5.7) for normal full-term pregnancies. Moreover, the standard error of the difference between these means is 11.5 giving $t = 4.1$ for $n = 52$. It should be pointed out that in calculating the standard error of the normal pregnant group the value of 387 μg./100 ml. has been included in the series, even though this differed from the mean by more than three times the standard deviation of the mean.

From the results which we have obtained on this small series of cases it would appear that in pre-eclampsia the serum copper level shows a small but statistically significant increase over the normal elevation occurring in pregnancy.

In order to obtain information as to the physiological significance of this extra serum copper in pregnancy it was decided to carry out some initial experiments to determine, by means of fractional precipitation of the serum proteins, whether this excess copper in the serum behaves in a similar fashion to the copper present in the serum of non-pregnant subjects, and also to obtain evidence as to which of the main groups of protein the copper is combined.

Total globulins were first precipitated with 26.8% Na_2SO_4 (Majoor, 1947; Milne, 1947), and the percentage of the total serum copper precipitating with the globulins determined. In these fractionation experiments advantage was taken of the finding by Tompsett (1934) that the serum copper can be quantitatively estimated on trichloroacetic acid filtrates of whole serum, acidification releasing the copper from its bound form.

The globulins, precipitated from 3 ml. of serum by means of 26.8% Na_2SO_4 (previously washed with ammoniacal sodium diethyldithiocarbamate, extracted with amyl alcohol, recrystallized and dried in the oven at 110° C.), were therefore dissolved in H_2O and re-precipitated with a final concentration of 5% trichloroacetic acid; after standing for 10 minutes the precipitate was centrifuged, and the supernatant transferred to a separating cylinder for copper estimation. The precipitate was washed once with 3 ml. 5% trichloroacetic acid, and the supernatant also added to the separating cylinder. Total serum copper was estimated on the filtrate obtained by precipitation of 3 ml. serum with trichloroacetic acid in the same manner.

In addition, Milne (1947) has shown that 19.6% Na_2SO_4 yields a precipitate of "euglobulin" which appears to correspond reasonably quantitatively with electrophoretically estimated β- + γ-globulins, and Kibrick and Blonstein (1948) have claimed that a fraction agreeing satisfactorily with electrophoretically estimated γ-globulin can be obtained by precipitation with 15% Na_2SO_4. An attempt was made therefore to estimate the copper in fractions precipitated with 15%, 19.6%, and 26.8% Na_2SO_4 in the hope of being able thereby to determine roughly the percentage of the total serum copper bound with the α-, β-, and γ-globulin fractions respectively. Some difficulty was experienced, however, in obtaining satisfactory flocculation for either filtration or centrifugation of the precipitates obtained at these salt concentrations without the use of high dilutions of the serum which would have introduced inaccuracies into the subsequent copper estimations. For this reason ether was added in order to obtain a compact and centrifugable separation of the precipitated globulins (Kingsley, 1940).

The procedure finally adopted was as follows: Serum, 3 ml., is added slowly to the weighed quantity of solid copper-free Na_2SO_4 contained in a round-bottomed centrifuge-tube which is stirred mechanically and immersed in a water-bath at 35° C. After stirring for five minutes the tube is removed from the bath, 1 ml. of ether added, and the tube shaken for 20 to 30 seconds. It is then immediately centrifuged for 10 minutes at 3,000 revolutions per minute. The ether is then poured off, and the lower aqueous layer removed with a teat-ended capillary pipette and discarded. The precipitate is dissolved in 4 ml. of water and re-precipitated with trichloroacetic acid as described above.

The percentages of the total serum copper precipitating with the various globulin fractions obtained by these means are shown in Table III. Using only Na_2SO_4 as the precipitating agent it will be seen that 89% of the copper present in the serum from non-pregnant subjects appears in the precipitate of total globulins; when Na_2SO_4 together with ether was used, the globulin precipitate contained 86% of the total copper present in the serum, a value not differing significantly from that obtained with Na_2SO_4 alone. A few experiments were also done to compare the effects of the use of ether on the fractions precipitating with 15% and 19.6% Na_2SO_4, but again it was found that the presence of ether did not affect the amount of copper precipitating at these concentrations. Although no electrophoretic study of these protein fractions has been made, it has therefore been concluded that we may justifiably consider these fractions as representing roughly the α-, β-, and γ-globulin fractions described by Kibrick and Blonstein (1948).

TABLE III

PERCENTAGES OF TOTAL SERUM COPPER PRECIPITATING WITH VARYING GLOBULIN FRACTIONS

Subjects	Precipitant			
	Na_2SO_4(%) + Ether			Na_2SO_4 (%)Alone
	15	19.6	26.8	26.8
Normal, non-pregnant	33	82	75	94
	21	79	97	95
	38	74	85	86
	16	81	86	86
	23	68	89	86
Mean % of copper ..	26	77	86	89
Pregnant (37–40 weeks)	31	84	98	
	29	81	94	
	22	80	100	
	22	76	85	
	25	81	100	
	24	—	86	
	22	—	90	
Mean % of copper ..	25	80	93	

The results summarized in Table III show that no significant difference exists between non-pregnant and pregnant sera as regards the amount of copper appearing in the protein fractions precipitated by 15%, 19.6%, and 26.8% Na_2SO_4. Further, using Kibrick and Blonstein's interpretation of these protein fractions, it appears that approximately 25% of the copper is combined with the γ-globulin fraction, 55% with the β-globulins, and a further 10% with the α-globulins.

Discussion

The considerable increase in the serum copper level in pregnancy observed by earlier workers has been confirmed. The main object of the present work was to discover whether this elevated serum copper level undergoes any change if a pre-eclamptic toxaemia develops in the course of the pregnancy. Although the number of cases of pre-eclamptic toxaemia which we have been able to study is small, the results show that in this condition there appears to be a small but statistically significant increase in the serum copper level over

the normal elevation which occurs in a healthy pregnancy. From the few cases we have studied there does not appear to be any obvious evidence of a correlation between the severity of the toxaemia and the height of the serum copper level, although further work on a larger series of cases would be needed to establish this point. It is clear, however, that the decrease in histaminolytic activity of the plasma in pre-eclamptic toxaemias, which has been reported by Kapeller-Adler (1944, 1947), is not accompanied by any corresponding fall in the level of serum copper.

With a view to obtaining information about the state and physiological significance of the raised serum copper in pregnancy, information has been obtained as to relative amounts of copper bound to the various fractions of the plasma proteins. Our finding that 90% of the serum copper appears in the globulin precipitate produced by 26.8% Na_2SO_4 agrees well with the proportion precipitated by 50% saturation with $(NH_4)_2SO_4$ (Holmberg and Laurell, 1947). By using graded concentrations of Na_2SO_4 it has been shown that the greater part of this globulin-bound copper exists in combination with those globulins precipitating between 15% and 19.6% Na_2SO_4, that is with a fraction which has been claimed by Kibrick and Blonstein (1948) to consist largely of β-globulins. In this connexion it is of interest that Longsworth, Curtis, and Pembroke (1945) have shown by electrophoretic analysis that in pregnancy there is a decrease in the serum albumin level accompanied by an approximately two-fold increase in the β-globulin level. Novak and Lustig (1947) have also reported a significant fall in the albumin/globulin ratio of the serum in pregnancy, and have claimed that this ratio is still further reduced in toxaemic patients, a finding which accords with our demonstration of a further rise in the serum copper level in pre-eclamptic toxaemias. Moreover, a β-globulin has recently been isolated from human plasma which is capable of combining with iron, copper, or zinc, and has been designated the "metal-combining globulin" (Cohn and Koechlin, 1947; Surgenor, Koechlin, and Strong, 1949). The properties of this metal-combining protein have so far mainly been studied in connexion with its combination with iron. Although the present findings are not concerned specifically with this protein, they indicate that not only does the larger part of the copper normally present in human serum also exist in combination with a β-globulin, but that the extra copper present during pregnancy appears to show the same distribution among the major fractions of the plasma proteins. Our results with human serum are therefore in contrast with the findings of Eisler, Rosdahl, and Theorell (1936) that the copper in horse serum is largely associated with the albumin fraction.

Summary

Estimations of the serum copper level have been carried out in blood samples obtained from healthy, non-pregnant women, from women late in pregnancy, from women four to eleven weeks post-partum, and in 12 cases of pre-eclamptic toxaemia.

The results indicate that in pre-eclampsia the serum copper level shows a small but significant increase over the normal elevation occurring in pregnancy.

Approximately 90% of the serum copper appears in the globulin precipitate produced by 26.8% Na_2SO_4.

By the use of graded concentrations of Na_2SO_4 the copper partition among various globulin fractions has been determined.

No significant difference was observed between samples from pregnant and non-pregnant subjects in the distribution of the protein-bound copper.

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