Influence of Estrogens on Total Serum Copper and Caeruloplasmin.* (22512)

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Studies in this laboratory involving the separation of low-density lipoproteins from Cohn Fraction I + III of human plasma have led to observations on the copper-bound protein, caeruloplasmin.

Methods. Plasma of human subjects, before and after a course of estrogen therapy, was fractionated according to method No. 10 of Cohn and his associates(1) as adapted in this laboratory(2). Fraction I + III containing all of the low-density lipoproteins, almost all of the caeruloplasmin, fibrinogen and trace amounts of other proteins, was then brought to a solution density of 1.063-1.070 with NaCl and centrifuged at 30,000 rpm in a Spinco Model L centrifuge for 21 hours at 5°C. All lipoproteins were thus floated to the top in a characteristic yellow, fatty layer while the remaining proteins were densely packed in a blue to blue-green layer in the bottom of the tube. The blue color was attributed to caeruloplasmin, and its intensity to the concentration of the copper-bound protein. It was consistently noted that the blue color of this layer in Fraction I + III from equivalent amounts of plasma was always more intense after subjects had received estrogen. Thus we were prompted to make quantitative measurements for copper and caerulopasmin before and after the hormone was given. Realizing the probability that caeruloplasmin was present in Fraction IV + V + VI as well as Fraction I + III of the Cohn method number 10 and that recoveries of minute amounts might not be ideal, analyses for copper were carried out on unfractionated serum. The diethyldithiocarbamate colorimetric method of Gubler and co-workers(3,4) was employed. With their procedure they demonstrated that all copper not complexed as caeruloplasmin reacts directly with the carbamate. This is termed the "direct" reacting fraction and normally accounts for only about 0-10% of the total serum copper. The "indirect" reacting fraction, or that portion not reacting directly with carbamate, presumably corresponds to caeruloplasmin and normally represents the bulk (90-100%) of the total copper. Markowitz, Gubler, Mahoney, Cartwright and Wintrobe (5) showed that concentrations of caeruloplasmin calculated from the copper values of the "indirect" fraction were in good agreement with those they obtained from immunochemical and Warburg manometric technics using paraphenylenediamine as substrate.

Results. Table I shows the results for total, "direct" and "indirect" copper concentrations in unfractionated serum from a variety of patients before and after a course of estrogen. It includes diagnoses and also dose and duration of estrogen treatment. None of the subjects had received any other form of drug therapy, either immediately preceding or during the experiments. None suffered respiratory infections. In all instances there was, after 3 to 4 weeks on 1 mg Estinyl (Ethinyl Estradiol)† o.d., a 1.5 to 2.5-fold increase in both the total and "indirect" reacting copper. Subject *Bla* still showed a 71% increase even after his dose had been lowered to 0.25 mg o.d. for 4 weeks. For purposes of comparison we have shown in the Table copper concentrations of normal males and females(6). Values in our laboratory on 6 normal individuals fell within their normal range.

Hypercupremia has been reported in a variety of diseases for which there is no known common denominator(7). The highest values reported appear to be in acute leukemia and in normal pregnant women(7). Higher values for total and "indirect" reacting serum

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TABLE I. Direct and Indirect Reacting Serum Copper Levels following Estrogen Therapy.

Subjects	Control (copper, γ/100 ml) Total	Direct	Indirect	Estrogen therapy‡ (copper, γ/100 ml) Duration (wk)	Dose (mg, o.d.)	Total	Direct	Indirect
Bla ♂ 39 coronary occlusion	157	25	132	4	1.0	334	52	282
				4	.5	283	77	205
				4	.25	268	47	221
Adi ♂ 49 coronary occlusion	153	15	138	4	1.0	356	34	322
				8	1.0	375	71	304
				15	1.0	522	35	487
Unr ♀ 47 myxedema	131	14	117	3	1.0	340	36	304
Cit ♀ 59 myxedema	254	34	220	4	1.0	374	18	356
Bor ♀ 72 myxedema	150	13	137	2	1.0	202	7	195
Cop ♀ 39 xanthomatosis	152	6	146	4	1.0	325	69	256
				15	1.0	320	93	227
Sta ♀ 49 xanthomatosis	175	24	151	4	1.0	328	29	299
Haa ♀ 56 xanthomatosis	169	0	169	8	1.0	360	39	321
				16	1.0	334	25	309
6 normals (ours)	(101–137)							
*23 normal ♀	116 ± 16							
40 " ♂	105 ± 16							
†10 mixed normals	109 ± 17	5 ± 6	103 ± 11					
10 normal pregnant ♀	257 ± 38	29 ± 13	228 ± 35					

* Reference (6). † Reference (5). ‡ Estrogen given as Estinyl (ethinyl estradiol).

copper were obtained after administration of estinyl than was found in any previously reported condition and even in the third trimester of pregnancy(5). In view of these observations and reports that serum copper concentrations are significantly higher in females than in males(6) the possible interrelationship between the enzymatically active caeruloplasmin and estrogen metabolism is suggested.

Summary. 1. Administration of estrogen as Estinyl (Ethinyl Estradiol) in doses ranging from 0.25-1.0 mg o. d. for 3 to 4 weeks markedly increased the serum total and "indirect" reacting copper or caeruloplasmin levels in 8 subjects with a variety of diseases. 2. Values obtained after 4 weeks on 1 mg Estinyl o. d. were considerably higher than in any previously reported conditions.

1. Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Derouaux, G., Gillespie, J. M., Kahnt, F. W., Lever, W. F., Liu, C. H., Mittelman, D., Mouton, R. F., Schmid, K., and Uroma, E., *J. Am. Chem. Soc.,* 1950, v72, 465.

2. Russ, E. M., Eder, H. A., and Barr, D. P., *Am. J. Med.,* 1951, v11, 468.

3. Gubler, C. J., Lahey, M. E., Ashenbrucker, H., Cartwright, G. E., and Wintrobe, M. M., *J. Biol. Chem.,* 1952, v196, 209.

4. Gubler, C. J., Lahey, M. E., Cartwright, G. E, and Wintrobe, M. M., *J. Clin. Invest.,* 1953, v32, 405.

5. Markowitz, H., Gubler, C. J., Mahoney, J. P., Cartwright, G. E., and Wintrobe, M. M., *ibid.,* 1955, v34, 1498.

6. Lahey, M. E., Gubler, C. J., Cartwright, G. E., and Wintrobe, M. M., *ibid.,* 1953, v32, 322.

7. Lahey, M. E., Gubler, C. J., Cartwright, G. E., and Wintrobe, M. M., *ibid.,* 1953, v32, 329.

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