Copper metabolism in retinitis pigmentosa

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Copper is widely distributed in nature and forms a constituent of most foodstuffs. An average daily diet of an adult contains about 2 to 5 mg of copper, and a large part of this ingested copper is excreted in faeces, its excretion through urine being negligible. According to Cartwright (1950) an adult human body contains a total of 100 to 150 mg of copper, and a major proportion of it is in mammalian plasma and circulates as caeruloplasmin. Scheinberg (1966) believed that caeruloplasmin played a role in the maintenance of zero copper balance in the body.

There appears to be a close correlation between copper and cellular activity in all living beings. It is known that an important connexion between copper and pigment formation exists (Underwood, 1956). As primary retinitis pigmentosa is apparently caused by a retinal pigmentary disturbance, a study of copper metabolism in patients suffering from this disease was undertaken. No report of such a study is available in the literature. In this paper the results of copper concentration in serum, caeruloplasmin concentration in plasma, and copper excretion in urine are reported and their possible interrelationship in the context of this disease is considered.

Material and methods

Fifteen patients with primary retinitis pigmentosa between the ages of 10 and 42 years were investigated. All of them belonged to middle class society eating a predominantly vegetarian mixed Indian diet. The diagnosis of all these cases was based on the positive results of the following parameters:

HISTORY

Each of these patients had a history of progressively increasing night blindness which had started early in the first decade. The night blindness did not respond to any conventional treatment and it was gradually followed by visual disturbances during the day. Some of the patients also had a positive family history.

FUNDUS PICTURE

A typical atrophic pale disc, attenuation of blood vessels, and pigment dispersal could be seen in all patients

except two in whom there was only generalized mottling of the fundus (Table I).

VISUAL ACUITY AND VISUAL FIELDS

Six patients had visual acuities ranging from 6/9 to 6/24 with or without correction while in nine the visual acuity was 6/60 or less. Perimetry showed moderate to marked generalized constriction of fields in most of them, but one patient showed a ring scotoma and in another fields were within normal limits. In others perimetry was not possible because of poor vision (Table I).

ELECTRO-OCULOGRAPHY

Electro-oculography was performed according to the method of Gahlot and Hansen (1974), and each patient was found to have a very low ratio. In five electro-oculograms were not possible as the patients could not see the fixation light during the dark phase (Table I).

ELECTRORETINOGRAPHY

Electroretinographic responses, both scotopic and photopic, were recorded and it was found that in five cases these were subnormal, while in the remaining 10 they were flat (Table I).

OTHER TESTS

None of these patients had a positive history of any systemic infection and their reactions to tests for syphilis and tuberculosis were negative. Their erythrocyte sedimentation rates were normal and the urine did not show albumin, sugar, or acetone. These patients were subjected to the following biochemical tests:

All fifteen cases of primary retinitis pigmentosa (11 males and 4 females) were selected to determine their level of total serum copper. This was done using the method of Eden and Green (1940) and Ventura and King (1951) as modified by Varley (1975). Ten normal subjects were included as controls.

The caeruloplasmin concentration levels in plasma using the method of O'Brien and Ibbott (1964), a modification of Ravin (1961), were established by determining the copper oxidase activity in 10 patients (7 males and 3 females) and in 10 normal people as controls (Tables II and III). All glass instruments were thoroughly demineralized and all of them except the blood collection tubes were siliconized. The clear plasma was stored at 4°C before analysis but never for more than one week. The haemolysed sample was discarded.

Table I Clinical and electrophysiological parameters for the diagnosis of retinitis pigmentosa

Patient no.	Age (years)	Sex	Visual acuity		Electroretinography	Electro-oculography ratio		Visual fields	Fundus
			Right eye	Left eye		Right eye	Left eye		
1	21	Male	PL PR	PL PR	Subnormal	Not possible		Not possible	Pale, blood vessels attenuated, pigment ++
2	20	Female	6/9	6/9	Flat	115	110	Tubular	Pale, blood vessels attenuated, pigment ++
3	25	Male	6/36 =6/24	6/24 6/12	Flat	125	125	Tubular	Pale, blood vessels attenuated, pigment ++
4	25	Male	6/60 =6/9	6/36 6/9	Subnormal	100	115	Generalized constriction	Pale, blood vessels attenuated, pigment ++
5	25	Female	2/60	2/60	Flat	100	100	Tubular	Pale, blood vessels attenuated, pigment ++
6	28	Male	6/9	6/9	Subnormal	112	135	Generalized constriction	Pale, blood vessels attenuated, pigment ++
7	15	Male	PL PR	PL PR	Flat	Not possible		Not possible	Pale, blood vessels attenuated, pigment ++
8	21	Male	6/18	6/18	Flat	110	100	Generalized constriction	Pale, blood vessels attenuated, pigment ++
9	34	Female	6/60 =6/24	6/60 6/24	Subnormal	110	120	Ring scotoma	Pale, blood vessels attenuated, pigment ++
10	42	Female	6/60	6/60	Flat	100	100	Generalized constriction	Pale, blood vessels attenuated, pigment ++
11	22	Male	CF 1m	6/60	Flat	115	120	Generalized constriction	Pale, blood vessels attenuated, pigment ++
12	25	Male	CF ½m	CF ½m	Flat	Not possible		Not possible	Pale, blood vessels attenuated, generalized mottling No pigmentation
13	42	Male	CF 1m	CF 1m	Flat	110	110	Not possible	Pale, blood vessels attenuated, pigment ++
14	10	Male	PL PR	PL PR	Flat	Not possible		Not possible	Pale, blood vessels attenuated, generalized mottling
15	22	Male	CF 2m	CF 2m	Subnormal	Not possible		Normal	Pale, blood vessels attenuated, pigment ++

Flat — Extinguished; = — Improves to; PL — Perception of light; PR — Projection of rays; CF — Counting fingers

Copper excretion in urine was determined using the method of Gubler, Lahey, Aschenbrucker, Cartwright, and Wintrobe (1952) as modified by Henry (1968). Ten patients (8 males and 2 females) and five normal subjects were included (Tables IV and V).

Observations

SERUM COPPER CONCENTRATION

The results showed that the concentration of total copper in the serum of normal subjects varied from 60 to 150 µg/100 ml of serum, 97.7 ± 27.13 ($n = 10$). However, the copper concentration in patients with retinitis pigmentosa varied from 36 to 108 µg/100 ml of serum, 77.6 ± 22.89 ($n = 15$). This showed that the mean figure in these patients was not significant at 5 per cent level ($P \geq 0.08$) although individually some of them had figures lower than normal.

PLASMA CAERULOPLASMIN CONCENTRATION (Tables II and III)

The caeruloplasmin in optical density units in normal subjects ranged from 0.19 to 0.31 (mean 0.23) while that in patients ranged from 0.15 to 0.03 (mean 0.08). Converting the average figures of optical density units in terms of caeruloplasmin concentration in mg (Ravin, 1961) gives 20.12 mg/100 ml for normal subjects and 7.0 mg/100 ml for patients. This showed that the concentration of caeruloplasmin in the plasma of patients with retinitis pigmentosa was significantly lower ($P < 0.001$).

COPPER EXCRETION IN URINE (Tables IV and V)

The results show a striking and statistically significant increase in urinary excretion of copper in patients with retinitis pigmentosa compared with normal subjects ($P < 0.001$).

Table II Plasma caeruloplasmin concentration in optical density units in normal subjects

Subject no.	Age (years)	Sex	Caeruloplasmin
1	26	Female	0.19
2	32	Female	0.31
3	22	Female	0.22
4	35	Female	0.19
5	32	Male	0.20
6	26	Male	0.28
7	40	Male	0.21
8	30	Male	0.21
9	25	Male	0.20
10	27	Male	0.31

Mean = 0.23; SD = 0.049

Table III Plasma caeruloplasmin concentration in optical density units in patients with retinitis pigmentosa

Patient no.	Age (years)	Sex	Caeruloplasmin
1	25	Male	0.15
2	25	Female	0.09
3	10	Male	0.06
4	15	Male	0.07
5	21	Male	0.06
6	25	Male	0.08
7	34	Female	0.07
8	42	Male	0.03
9	42	Female	0.13
10	10	Male	0.07

Mean = 0.08; SD = 0.035; $P < 0.001$ highly significant

Discussion

The plasma concentration of copper in Americans and Europeans is about 100 µg/100 ml of plasma (range 75 to 160 µg/100 ml) (Gubler, 1956; Markowitz, Gubler, Mahoney, Cartwright, and Wintrobe, 1955; Underwood, 1956; Varley, 1975). This corresponds well with the current figures for normal Indian subjects. Blood contains two principal fractions of copper. About 6 per cent is loosely bound to serum albumin while 94 per cent is tightly bound to serum globulin as caeruloplasmin fractions. When copper first enters the plasma from the gastrointestinal tract it is believed to be loosely bound to albumin and is gradually transferred in the liver to caeruloplasmin. Some is present as free cupric ion which is in equilibrium with copper loosely bound to albumin. The loosely bound copper is probably the portion being transferred because unlike copper bound to caeruloplasmin it can diffuse freely across semipermeable membrane

and appears in urine. From plasma, copper goes to several sites in the body and exists in many forms *in vivo*, the total function of which is not yet known.

Copper metabolism seems peculiarly susceptible to alterations in a wide variety of clinical conditions. Many conditions are accompanied by a marked increase in plasma copper such as most chronic infections, myocardial infarction, hyperthyroidism, hepatitis, post-hepatitis cirrhosis, portal cirrhosis, iron deficiency anaemia, hypoproteinaemia, pellagra, chronic alcoholism, schizophrenia, Hodgkin's disease, scleroderma, leukaemias, and carcinoma (Scheinberg and Sternlieb, 1960). Like erythrocyte sedimentation rate and white blood corpuscle count, serum copper is an acute phase reactant and is probably related to the general metabolic state of the tissue. The condition of hypercupraemia is usually associated with a corresponding rise in caeruloplasmin concentration because a major part of copper exists in the body as a component of caeruloplasmin. However, the exact relationship is not clear. The occurrence of low serum copper levels is less frequent than high levels. Hypocupraemia in humans occurs in new-born infants, sprue, nephrotic syndrome, and hepatolenticular degeneration (Scheinberg and Sternlieb, 1960). It appears that hypocupraemia is caused by a decrease in the concentration of caeruloplasmin.

Table IV Copper excreted in the urine of normal subjects in 24 hours

Subject no.	Age (years)	Sex	Urine (µg/24h)
1	30	Male	11
2	40	Male	38
3	29	Male	46
4	23	Male	40
5	24	Male	25

Mean = 32 µg/24h; SD = 14.02

Table V Copper excreted in the urine of patients with retinitis pigmentosa in 24 hours

Patient no.	Age (years)	Sex	Urine (µg/24h)
1	45	Male	156
2	25	Male	170
3	35	Female	169
4	10	Male	213
5	15	Male	260
6	25	Male	222
7	8	Male	200
8	12	Female	180
9	42	Male	151
10	22	Male	240

Mean = 186.1 µg/24h; SD = 54.28; $P < 0.001$ highly significant

Caeruloplasmin is an alpha-globulin and accounts for most of the copper ions and oxidase activity of blood which in turn varies with the serum copper levels. The exact nature of physiological function of caeruloplasmin is not known except that, being a member of the copper enzyme system in the body, it is in some way related to the oxidative processes of the tissue, probably at the mitochondrial level.

No report is available in the literature in which the copper metabolic state in patients with primary retinitis pigmentosa has been studied and, therefore, no figures are available on their serum copper and caeruloplasmin concentrations or their copper urinary excretion. The observations in our study show that all the patients had either normal or low serum copper concentration although the average figure was not significantly lower than normal. This normal or near normal figure was associated with a highly statistically significant low copper oxidase activity—that is low caeruloplasmin concentration. Furthermore, in our series all the patients showed a very greatly increased excretion of copper in the urine.

The amount of copper excreted in normal human urine varies considerably but the different techniques used with the possibility of contamination with extraneous copper may contribute to the divergent results. Rabinowitch (1933) reported from 'traces' to $700 \mu\text{g}/24\text{h}$ while Munch-Peterson (1950) and Keltz (1959) found 120 to $520 \mu\text{g}/24\text{h}$ for adults and 20 to $90 \mu\text{g}/24\text{h}$ for children. Van Revesteyn (1944) reported 0 to $100 \mu\text{g}/24\text{h}$ while Heilmeyer, Keilerling, and Stuwe (1941) could find only 'the most minimal traces' of copper in urine. The preponderance of recent evidence, however, indicates that urinary copper excreted is about 0 to $30 \mu\text{g}/24\text{h}$ (Porter, 1951; Mahoney, Bush, Gubler, Moretz, and Cartwright, 1955; Butler and Newman, 1956). The output is considerably higher if there is concomitant proteinuria (Uzman and Denny Brown, 1948). Chatterjee and Ganguly (1950) observed that an Indian excreted less copper in urine than a European probably because of differences in diet.

An absolute magnitude of urinary copper excretion is of special interest since increased urinary excretion of copper is seen only in hepatolenticular degeneration (Mondelbrote, Stainer, Thompson, and Thruston, 1968; Porter, 1949; Cumings, 1951) and nephrotic syndrome with albuminuria (Cartwright and Gubler, 1954).

Munch-Peterson (1950) analysing urinary excretion in nephritis and other conditions causing proteinuria found a rough linear correlation between amounts of copper and protein excreted. It appears that an increase in urinary copper in association

with proteinuria could be accounted for by an abnormal leak through the glomeruli of the copper protein found in the plasma. This cannot account for the small amount of excreted copper found in normal subjects nor for the greatly increased excretion in hepatolenticular degeneration. In retinitis pigmentosa this is still more difficult to explain because our preliminary investigations showed that there was no gross deficit in the kidney functions in patients nor was there any significant proteinuria.

Thus our series of patients of primary retinitis pigmentosa show a triad of interesting findings:

Normal or near normal serum copper concentration,

Significantly low caeruloplasmin concentration,

High urinary copper excretion.

These findings are important because they have so far not been reported and this combination of findings is a feature of chronic copper toxicity as seen in hepatolenticular degeneration. Liver biopsy studies conducted in some cases of retinitis pigmentosa have also shown a few mild non-specific changes indicating that in primary retinitis pigmentosa copper metabolism may also be abnormal.

An attempt was made at producing a condition similar to retinitis pigmentosa in monkeys and rabbits by injecting them with a copper sulphate solution, and details will be published later. The condition histologically appears to simulate some feature reported for sodium iodate and iodacetate indicating that copper may be acting in the same manner as these toxic substances. Copper is a strong inhibitor of the sulphhydryl group which is an essential cofactor in the metabolic pathways of glycolysis and necessary for the regeneration of visual purple.

The clinical importance of such an hypothesis is obvious. If it is true, the use of a chelating agent—such as penicillamine—may gradually improve the condition. On this assumption one of us (DKG) actually began treatment on two patients in this series although the follow-up of a few months is too short to judge the response. It thus appears that retinitis pigmentosa may not be an abiotrophy but a condition of chronic copper toxicity due to an inborn error of copper metabolism.

Summary

Clinically and electrophysiologically confirmed cases of primary retinitis pigmentosa have been investigated regarding their copper metabolic state. It is observed that these patients show a normal or near normal serum copper concentration, very low plasma caeruloplasmin concentration, and a very high copper urinary excretion. A similarity between

this condition and hepatolenticular degeneration is drawn and it is suggested that retinitis pigmentosa may also be a condition caused by an inborn error of copper metabolism.

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