Copper Content of Hair in Normal Families and those with Wilson's Disease

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Despite much recent interest in the abnormality of copper metabolism in Wilson's disease (hepatolenticular degeneration) (Walshe and Cummings, 1961) there is surprisingly little published material concerning the copper content of the hair in this condition. We have traced only two publications dealing specifically with this aspect of the disease. In the first, Rice and Goldstein (1961) examined 18 hair samples from 10 patients (6 male and 4 female) and found an average hair copper content of 11.6 $\mu\text{g./g.}$ which was lower than the figure they found for their controls, 15.9 $\mu\text{g./g.}$ in males and 39.0 $\mu\text{g./g.}$ in females. There appeared to be no significant fall in the hair copper of patients with Wilson's disease following prolonged treatment with penicillamine. In the second published series, Martin (1964) studied only 4 patients and found the mean copper content was 15.2 $\mu\text{g./g.}$, again slightly less than in the control subjects, 16.0 $\mu\text{g./g.}$ for males and 19.5 $\mu\text{g./g.}$ for females. The close relatives of Wilson's disease patients were also in the normal range. We have extended these studies on a larger series of patients and controls correlating the hair colour with its copper content, and we have also studied families as complete units where possible.

Methods and Subjects

All hair samples were washed three times with methyl alcohol and twice with de-ionized water before oven-drying. Accurately weighed aliquots of approximately 100 mg. were wet-ashed with 1 ml. sulphuric and 1 ml. perchloric acid. The resultant clear solution was neutralized with 15 ml. 25% ammonium hydroxide; to this was added 1 ml. 4% sodium pyrophosphate and 1 ml. 0.1% sodium diethyldithiocarbamate. The coloured copper dithiocarbamate complex was extracted with 5 ml. amyl alcohol and estimated in a spectrophotometer at 440 m μ . Suitable blanks and standards were run with each set of determination.

Hair samples were obtained from 24 patients with Wilson's disease. All these had the classical disorder

of copper metabolism and all but 3 had Kayser Fleischer rings: these three were younger sibs of clinically manifest patients who were identified by chemical and isotope studies carried out routinely on all families known to carry the Wilson's disease gene. In addition 28 parents of these patients were studied, also 11 sibs and 2 children. In the Table of results these are all classified as first degree relatives, as there was no significant difference between the groups. Finally our control subjects consisted of 40 laboratory staff and their close relatives (20 male and 20 female).

Results

The results are summarized in the Table; it is at once obvious that there is no significant difference between the normals, the patients with Wilson's disease, or their near relatives. However, in all groups the females have a slightly higher hair copper concentration than the males and further the fair-haired individuals (including red hair) have a higher copper content than those with medium brown hair, and these in turn were found to have higher values than those with dark brown or grey hair. One control subject with naturally very fair hair and the mother of a patient with Wilson's disease with medium brown hair were found to have unusually high values (62.5 and 146.0 $\mu\text{g./g.}$, respectively); further hair samples from these two subjects were reassayed and the results confirmed (72 and 110 $\mu\text{g./g.}$).

Four family trees are shown (Fig. 1-4) with the copper content and hair colour for each individual appropriately marked.

In the first normal family, comprising 18 persons spread over 3 generations, the mean hair copper content was 10.1 $\mu\text{g./g.}$ which is lower than the mean for the control group as a whole (16.4 $\mu\text{g./g.}$) and the range of variation for this family was 5.3 to 16.5 $\mu\text{g./g.}$ In the second normal family the mean copper content of the hair of 9 subjects was 18.4 $\mu\text{g./g.}$ and ranged from 11.3 to 24.8 $\mu\text{g./g.}$

In the first Wilson's disease family the mean hair copper was 14.9 $\mu\text{g./g.}$ and there was remarkably little variation between the patient (II. 2), his 7 sibs,

NORMAL 1.

FIG. 1.

NORMAL 2.

FIG. 2.

KEYHair Colour

D Dark
M Medium
F Fair
G Grey

Wilson's Disease gene

● Homozygous abnormal
◐ Heterozygous normal
○ Homozygous normal
⊖ Not known

WILSON'S DISEASE 1. WILSON'S DISEASE 2.

FIG. 3 and 4.

FIG. 1-4. Family trees of two normal and two Wilson's disease families. The copper concentration on the hair is expressed as $\mu\text{g./g.}$ dry weight, the hair colour is indicated by the appropriate letter, D, M, F, G (see key) and the Wilson's disease gene by shading (see key). The heterozygotes in Wilson's disease family I are identified by studies with ^{64}Cu . (See Walshe and Cumings (1961) p 141.)

TABLE
CONCENTRATION OF COPPER IN HAIR

		No. of Cases	Range ($\mu\text{g./g.}$)	Mean ($\mu\text{g./g.}$)	Fair			Medium			Dark			White		
					No. of Cases	Range ($\mu\text{g./g.}$)	Mean ($\mu\text{g./g.}$)	No. of Cases	Range ($\mu\text{g./g.}$)	Mean ($\mu\text{g./g.}$)	No. of Cases	Range ($\mu\text{g./g.}$)	Mean ($\mu\text{g./g.}$)	No. of Cases	Range ($\mu\text{g./g.}$)	Mean ($\mu\text{g./g.}$)
Normal	All	40	5.4-72.0	16.4	13	7.8-72.0	21.7	9	6.8-36.4	16.21	14	5.4-24.8	13.2	4	7.7-15.2	11.3
	Males	20	5.4-55.7	14.3	3	11.4-55.7	28.2	6	6.8-24.8	12.9	8	5.4-16.5	10.9	3	7.7-15.2	12.1
	Females	20	7.7-72.0	18.5	10	7.8-72.0	19.8	3	14.4-36.4	22.8	6	11.4-24.8	16.2	1		7.7
First degree relatives of Wilson's disease patients	All	41	7.5-146	21.8	4	23.8-33	30	23	10.6-146	23.6	13	7.5-55.5	17.1	1		10.8
	Males	23	7.5-40.0	17.6	3	23.8-33	28.9	12	10.6-40.0	18.3	7	7.5-15.5	12.5	1		10.8
	Females	18	11.1-146	27.3	1		33.0	11	13.1-146	29.3	6	11.1-55.5	22.6			
Wilson's disease patients	All	24	5.5-86.5	19.1				12	5.5-86.5	22.3	12	5.5-28	15.9			
	Males	11	5.5-32.5	17.6				4	12.5-32.5	19.9	7	5.5-27	16.3			
	Females	13	5.5-86.7	20.4				8	5.5-86.5	23.5	5	9.6-28	15.4			
All cases	All	105	5.4-146	18.2	17	7.8-67.5	23.6	44	5.5-146	21.7	39	5.4-55.5	15.4	5	7.7-15.2	11.3
	Males	54	5.4-55.7	16.5	6	11.4-55.7	28.5	22	6.8-40	17.1	22	5.4-27	13.1	4	7.7-15.2	12.1
	Females	51	5.5-146	22.1	11	7.8-67.5	20.9	22	5.5-146	26.3	17	9.6-55.5	18.2	1		7.7

and their father, but the mother's concentration of copper in the hair was significantly higher. The second Wilson's disease family comprised 5 subjects; the parents are first cousins and 2 of their 3 children (II. 1, 3) are homozygous for the Wilson's disease gene. In this family the girl had the same hair copper as her mother and the 2 boys took after their father, the mean for the 5 being 19.1 $\mu\text{g./g.}$

We have not been able to make extensive comparisons of the concentration of copper in the hair before and after prolonged treatment with penicillamine in the Wilson's disease patients, but the findings from one patient before treatment (11.7 $\mu\text{g./g.}$) can be compared with those made two years later (12.8 $\mu\text{g./g.}$) during which time she had been taking 450 mg. of D-penicillamine HCl twice daily. In addition we have analysed hair from 4 subjects who had only just started treatment; the mean value for these was 16.8 $\mu\text{g./g.}$ with a range from 10.2 to 23.9 $\mu\text{g./g.}$; this can be compared with a mean of 19.1 $\mu\text{g./g.}$ for the whole group of 24 Wilson's disease patients.

Discussion

In these investigations, using a relatively large series of patients with Wilson's disease and control subjects, we have confirmed earlier reports that there is no significant increase in the concentration of copper in the hair in Wilson's disease and that long-term treatment with penicillamine does not further deplete the hair copper, though there is a significant reduction in the plasma copper as a result of this therapy (Walshe, 1964; Walshe and Patston, 1965). We are also in agreement that

females have a slightly higher content of copper in their hair than males, and we have, as far as possible, tried to avoid the effect of hair-dressing preparations in making this assessment. We have made the additional finding that there is a higher concentration of copper in the hair of fair- as compared with dark-haired subjects. Estimations made on grey hair show that there is no increase in the content of the metal with ageing. It should be noticed that none of our Wilson's disease patients fell into the fair-hair group, and as the majority of patients were children or adolescents this observation may be of some significance. It is interesting to speculate what effect the excess non-caeruloplasmin copper in their plasma might have on the formation or activation of the copper protein tyrosinase which mediates the enzymatic conversion of tyrosine to melanin.

A study of the family trees shows that in the first normal family copper content of hair is uniformly low irrespective of hair colour, and this family might almost be said to carry a 'low hair copper' gene. In the second normal family the content of copper in the hair is uniformly higher, and III. 1 and 2 have remarkably similar values to their father. The similarity of the values for the 8 sibs in the larger of the Wilson's disease families has already been commented upon.

Summary

We have estimated the concentration of copper in the hair of 40 normal subjects, 24 patients with Wilson's disease, and 41 of their close relatives. There was no significant variation in the copper

content of these three groups nor did chelation therapy lower the concentration of copper in the hair of the patients.

When the three groups were added together we calculated that the mean copper content of hair in 54 males was 16.5 µg./g. with a range from 5.4 to 55.7 µg./g.; in 51 females the mean was 22.1 µg./g. and ranged from 5.5 to 146 µg./g. Those with fair hair were found to have a higher copper content than those with dark hair.

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