The copper content of the liver and hair of African children with kwashiorkor

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Kwashiorkor is a disease of young children living in tropical countries and is associated with a deficiency of protein and a relative excess of carbohydrate in the diet. During the course of the disease there is an increase in the lipid content of the liver, often accompanied by anaemia, and changes in the texture and pigmentation of the skin and hair (Trowell, Davies & Dean, 1954).

Recently Lahey, Behar, Viteri & Scrimshaw (1958) and Edozien & Udeozo (1960) reported a marked reduction of blood copper levels in kwashiorkor and suggested that changes in the blood and pigmentation in this condition might be related to a Cu deficiency. We have therefore studied the copper content of liver and hair in a number of African children with kwashiorkor to learn whether this disease produces alteration in the concentration of Cu in these tissues.

EXPERIMENTAL

Material. Samples of liver were obtained from seven South African children aged 1–3 years who had died from kwashiorkor. Samples were also obtained from nine children in the same age group who had died as a result of diseases other than kwashiorkor and these served as controls. The pieces of liver were dried in a vacuum desiccator over conc. sulphuric acid. The dried material was stored in small glass bottles fitted with plastic screw-cap tops and sealed with paraffin wax.

Portions of hair were cut from seven living children suffering from kwashiorkor and from five other children in Durban, placed in clean paper envelopes, and sealed.

Analytical methods. Throughout the analytical work great care was taken to avoid contamination with Cu. The glass apparatus used was made of borosilicate glass and was cleaned by immersion in a concentrated solution of hydrochloric acid overnight, followed by repeated washings with de-ionized water. Flasks used in the analytical procedure were further treated by boiling de-ionized water in them.

All reagents used in the work, with the exception of the ammonium citrate, were of A.R. quality. The A.R. amyl alcohol was found to contain appreciable quantities of Cu, which was removed by double distillation of the alcohol in glass-jointed apparatus. A 50% (w/v) aqueous solution of ammonium citrate was made up, and the Cu present in it removed by the addition of a 1% (w/v) aqueous solution of sodium diethyldithiocarbamate; the Cu complex formed was extracted with chloroform until the solvent layer remained colourless.

Fat extraction. The fat was removed from whole dry liver samples by extraction for 24 h with light petroleum (b.p. 40–60°) in a conventional glass-jointed Soxhlet apparatus. The solvent was removed with the aid of heat and reduced pressure and the fat content determined by weight.

Preparation of samples for determination of copper. The portions of dried liver with and without fat, which had been minced with scissors, were placed in clean weighed 50 ml Erlenmeyer flasks, with clean stainless steel instruments. The contents of the flasks were dried to constant weight at 110°. The procedure was similar for the hair samples.

Copper determination. The tissue samples were ashed and their Cu contents determined with sodium diethyldithiocarbamate (Eden & Green, 1940). The yellow complex formed in the presence of Cu was extracted into redistilled amyl alcohol and measured spectrophotometrically at 435 mμ.

RESULTS

Liver. The concentrations of Cu in the livers of African children with kwashiorkor and of those children from the same region who had died from diseases other than kwashiorkor are shown in Table 1. There was a highly significant reduction in the amount of Cu per g dried tissue in the livers of children with kwashiorkor compared with the values obtained from the control group ($P < 0.01$).

The mean percentage of fat in the livers of children with kwashiorkor was three

Table 1. *Comparison of the copper content of livers of African children expressed on a whole dry and a fat-free dry tissue basis*

Subject			Fat (g/100 g tissue)	Cu (μg/g dry tissue)		
				Fat-free tissue	Whole tissue	
Code	Age	Diagnosis			Determined	Calculated
C11	Unknown	Kwashiorkor	35·6	13·4	6·0	8·6
B14	1 year	Kwashiorkor	40·2	11·8	8·0	7·0
B6	Unknown	Kwashiorkor	54·9	22·3	7·7	10·0
C10	Unknown	Kwashiorkor	55·9	18·9	7·2	8·3
C19	Unknown	Kwashiorkor	67·9	15·9	6·6	5·1
B5	7 months	Kwashiorkor	71·0	21·2	4·7	6·2
B38	Unknown	Kwashiorkor	78·3	17·3	—	3·8
	Mean		57·7	17·3	6·7	7·0
	Standard deviation		—	± 3·9	± 1·2	—
LM20	3 years	Stenosis of small intestine	6·2	16·8	10·5	15·8
LM3	3 years	Tuberculosis	7·9	29·0	32·0	26·6
LM36	1 year	Malnutrition	9·0	20·4	—	18·6
LM34	3 years	Malaria	10·2	24·2	27·0	21·6
LM4	1 year	Malaria	12·3	20·5	14·4	17·9
B16	3 years	Patent ductus arteriosus	13·8	28·1	26·1	24·1
B13	1 year	Whooping cough	15·0	19·4	20·6	16·4
B12	9 months	Bronchopneumonia	35·9	21·4	12·6	13·7
LM7	1 year	Pleural effusion	61·5	40·6	10·4	15·6
	Mean		18·1	24·4	19·2	18·9
	Standard deviation		—	± 7·3	± 8·4	—

times as great as that for the control group. In order to investigate whether the significant difference between the groups shown in Table 1 was due to the differences in fat content, the fat was extracted from a portion of each liver and the concentration of Cu again determined.

The results (Table 1) show that there was significantly less Cu per g fat-free dry liver from children with kwashiorkor than there was in the livers of the controls ($P < 0.05$). In both groups there was good agreement between the values for mean Cu content of liver tissue determined by direct analysis and by calculation. This strongly suggests that there was no Cu present in the liver fat in either group.

Hair. Comparison of the Cu content of hair from South African children with and without kwashiorkor (Table 2) showed a significant difference between the two groups ($P < 0.05$).

Table 2. *Copper content of hair samples from African children*

Subject			
Code	Age	Diagnosis	Cu (μg/g dry hair)
M.M.	2 years	Kwashiorkor	13·7
Z.L.	Unknown	Kwashiorkor	13·1
M.N.	Unknown	Kwashiorkor	13·0
K.N.	2 years	Kwashiorkor	15·6
M.D.	10 months	Kwashiorkor	19·0
D.N.	2 years	Kwashiorkor	11·4
B.N.	2 years	Kwashiorkor	11·5
	Mean		13·9
	Standard deviation		± 2·7
G.Z.	Unknown	Tuberculosis	21·0
G.N.	2 years	Post-measles pneumonia	24·3
I.M.	6 months	Tuberculosis	14·6
J.N.	Unknown	Unknown	18·3
T.M.	1 year	Tuberculosis	14·4
	Mean		18·5
	Standard deviation		± 4·2

DISCUSSION

There are clinical features in common between the recognized Cu deficiency in sheep known as 'sway-back' and kwashiorkor. The more striking signs are discoloration and textural alteration of the wool or hair and anaemia with hypocupraemia. Though it is not considered that kwashiorkor is a Cu-deficiency state, it may be that one of the deficiencies that is common and may account for some of the physical signs in this 'pluricarential' syndrome is Cu, just as various vitamin shortages may also be concomitant. The hair in kwashiorkor is frequently fragile and a well-documented feature is the change in colour, sometimes to red. The finding of a reduced concentration of Cu in the hair in cases of kwashiorkor does not, of course, mean that this shortage is responsible for the appearance of the hair. However, copper is important in the enzymic conversion of cysteine to cystine and it may well be that the decrease in cystine in hair from children with kwashiorkor (Platt & Nagchaudhuri, 1954) is a reflection of the Cu deficiency. The fact that Cu is low in such hair means that its

chemical composition is altered, but this does not imply an alteration in the enzymic processes concerned with hair formation.

The liver plays an important part in Cu metabolism and it may be that there is a loss of Cu storage capacity. There is very little Cu in maize (0·06–0·08 mg/100 g fresh maize, Albritton, 1954), the staple diet of these people, but it is unlikely that this has been the cause of the Cu deficiency found because it has proved impossible to produce Cu deficiency in healthy children on a milk diet (Wintrobe, Cartwright & Gubler, 1953), and no case of Cu deficiency has been reported in man.

The total liver weights were not determined and therefore it is impossible to know if the reduced Cu concentration means any alteration in total liver Cu. However, as there is a marked protein deficiency in kwashiorkor the size of the fat-free portion of liver would almost certainly be less than normal, and this would therefore mean that there exists a reduction in total liver Cu in kwashiorkor.

SUMMARY

1. The concentration of copper in the liver and hair of seven African children suffering from kwashiorkor has been compared with the values obtained from nine children from the same area suffering from other diseases.

2. A significant reduction in the concentration of Cu in the livers of children with kwashiorkor, compared with the values for controls, was found when the results were expressed either on a whole dry-tissue basis or on a dry fat-free basis. Calculation showed no Cu to be present in liver lipid.

3. Samples of hair taken from South African children with kwashiorkor have been analysed for Cu and the results compared with those for hair taken from children suffering from other diseases. There was a significant reduction in the concentration of Cu in the hair in kwashiorkor.

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