Plasma Copper and Iron Levels and Plasma Paraphenylene Diamine Oxidase Activity (Plasma Copper Oxidase Activity) in Kwashiorkor

By B. REIFF AND H. SCHNIEDEN

Anemia is often present in kwashiorkor—a disease occurring mainly in children aged one to four years. It is now well established that this disease is associated with a deficiency of protein in the diet, and Altmann et al. have noted marked improvement in the anemia following high protein therapy. In 1948 Waterlow suggested that certain of his malnourished children who had an anemia resistant to iron therapy might suffer from a copper deficiency and in 1952 Stransky et al. reported low serum copper levels in malnourished infants and children in the Phillipines. More recently (1957, 1958) Lahey et al. have reported low serum copper levels in malnourished Guatemalan children. It therefore seemed of interest to see if the kwashiorkor syndrome in Nigeria was also associated with low serum copper levels and furthermore to determine the serum copper oxidase activity in such cases since this activity is known to be markedly reduced in hepatolenticular degeneration, a disease in which—as is well known—abnormal copper metabolism occurs.

Methods

A series of children (male and female) between one and four years old with kwashiorkor and a similar series of children without kwashiorkor (the control group) were investigated. All the children attended the Outpatient Department of the University College Hospital, Ibadan. The “controls,” who were children of the same age group as the children with kwashiorkor, showed neither evidence of malnutrition nor obvious clinical evidence of anemia and were attending the hospital for treatment for some minor ailment, e.g., a cold.

Plasma copper concentration was determined by the method of Gubler et al. and plasma iron concentration by the method of Hamilton et al. Hemoglobin and hematocrit were determined by Wintrobe’s method. Plasma volume (Evans blue space) was determined by the method of Chinard. Plasma paraphenylene diamine oxidase activity (copper oxidase activity) was estimated by the method of Ravin and total plasma protein concentration by the biuret method of Kingsley.

Blood films were made at the same time that blood was taken for plasma copper. The slides were stained with either Leishman’s or Wright’s stain. Blood was collected into heparinized tubes. Estimations showed that the copper and iron content of the heparin used (Liquemin, Roche, Ltd.) was negligible.

Results

Table 1 shows the mean figures obtained for plasma copper and iron concentrations, total plasma protein concentration, plasma paraphenylene diamine oxidase activity, hemoglobin concentration and hematocrit levels.

From the Department of Pharmacology, University College, Ibadan, Nigeria.
We are indebted to Drs. R. C. Hendrickse, S. Fitzgerald and M. Hill of the Department of Pediatrics, University College Hospital, Ibadan for permission to study their patients and for their help in the determination of Evans blue space; to Mr. D. Allen, Department of Pharmacology, University College, Ibadan for technical assistance; and to the West African Medical Research Council for a grant for research on malnutrition.
 Submitted June 25, 1958; accepted for publication Jan. 16, 1959.
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Kwashiorkor children (age 1-4 years) Mean ± S.E.</th>
<th>Non-kwashiorkor children (age 1-4 years) Mean ± S.E.</th>
<th>t and P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Copper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (µg. 100 ml.)</td>
<td>46.1 ± 4.1</td>
<td>90.7 ± 3</td>
<td>t = 30</td>
</tr>
<tr>
<td></td>
<td>(22)</td>
<td>(23)</td>
<td></td>
</tr>
<tr>
<td>Plasma Iron</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (µg. 100 ml.)</td>
<td>54.3 ± 5.9</td>
<td>87.4 ± 11</td>
<td>t = 7.0</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
<td>(9)</td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% RBC</td>
<td>33.6 ± 1.4</td>
<td>37.1 ± 1.1</td>
<td>t = 4.6</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin %</td>
<td>60.3 ± 3.2</td>
<td>72.2 ± 4.1</td>
<td>t = 7.1</td>
</tr>
<tr>
<td>100% = 14.6 Gm.</td>
<td>(18)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Total Plasma Proteins</td>
<td>4.29 ± 0.23</td>
<td>5.93 ± 0.36</td>
<td>t = 3.9</td>
</tr>
<tr>
<td>Gm. 100 ml.</td>
<td>(12)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Paraphenylene diamine oxidase activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(expressed as % transmission at 530 µm.)</td>
<td>39.4 ± 5.1</td>
<td>21.3 ± 1.6</td>
<td>t = 3.4</td>
</tr>
<tr>
<td>Blank = 100%</td>
<td>(12)</td>
<td>(12)</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Figures in parenthesis are number of children on which estimation was made.

It will be noted that the plasma copper and iron concentrations are much lower in patients having kwashiorkor than in the controls and so is the plasma paraphenylene diamine oxidase activity. The plasma protein level is, as expected, lower in the kwashiorkor patients.

Examination of the blood films of the children with kwashiorkor revealed a hypochromic, normocytic anemia. Anisocytosis occurred. No malarial parasites were noted in the blood films of either the children with kwashiorkor or the controls.

In four controls and four patients with kwashiorkor, plasma volume (Evans blue space) and plasma copper were determined and total circulating copper calculated. There was a significant difference between the total circulating copper in the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Controls Mean ± S.E. : 461 ± 18 µg.</th>
<th>Kwashiorkor patients Mean ± S.E. : 158 ± 11 µg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>16</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Our results confirm those of Stransky et al. and of Lahey et al. that in kwashiorkor, low levels of circulating copper can occur. Zipursky et al. have stated that hypocupemia may be due to (a) inability to synthesize ceruloplasmin or (b) to excessive loss or destruction of ceruloplasmin.

Regarding the first possibility, these authors further state that such a condition can arise from lack of copper or inability to synthesize the apoprotein.

The daily requirements of copper and iron for infants and children are approximately 50 µg./Kg. and 500 µg./Kg. body weight, respectively.
cassava, however, the staple food of the area, only contains approximately
140 µg. copper/100 Gm. and 1100 to 3500 µg. iron per 100 gm. Moreover, it has been estimated that only approximately 5 per cent of the copper in an ordinary diet is retained and iron absorption is of a similar relative order. If such is the case in children with kwashiorkor, it can be seen that it is extremely likely that the copper and possibly the iron intake will be below normal. For example, in the case of copper, a child weighing 10 Kg. would require 500 µg. of copper per day. If this comes solely from cassava, approximately 330 Gm. of cassava is necessary, even if there is no lack of absorption, and seven Kg. of cassava approximately if there is only 5 per cent absorption. While it is not contended that children with kwashiorkor live solely on cassava, it nevertheless forms a major part of their diet.

Moreover, malabsorption may also inhibit copper absorption. A history of diarrhea is often given by patients with kwashiorkor, and changes affecting the exocrine glands, for example the pancreas, also occur in such patients.

Nevertheless, the possibility that the low plasma levels for copper noted may be due to deficiency of the apoprotein cannot be ignored since in the pig it is known that chronic protein deficiency can reduce serum copper.

However, Lahey et al., who have recently published data on South American children with kwashiorkor, noted that though the serum copper level in these children was low, the average value obtained for a2 globulin for the malnourished children did not differ appreciably from normal values. Since ceruloplasmin is an a2 globulin, they considered that it was doubtful if the low serum copper in children with kwashiorkor was due simply to insufficient quantities of apoprotein. The total plasma protein concentration in our patients with kwashiorkor was similar to those found by Lahey et al. in their patients and hence, though the a2 globulin concentration was not measured in our patients, it is likely that similar results would have been obtained.

Holmberg and Laurell have shown that approximately 90 per cent of the copper in serum is ceruloplasmin and that ceruloplasmin is an enzyme capable of acting on a substrate of paraphenylenediamine. Moreover, this enzyme activity is associated with the copper-containing portion of ceruloplasmin and not with the colorless portion remaining after copper has been removed from ceruloplasmin by dialysis against potassium cyanide. The significant reduction in paraphenylenediamine oxidase activity found in the present work therefore shows either an inability to synthesize, or excessive destruction of, the active group of ceruloplasmin which contains copper.

Finally, whether increased excretion of copper occurs in kwashiorkor remains to be determined. However, it is known that copper is excreted in the bile, and it is therefore possible that diarrhea resulting in an excessive loss of biliary contents with resultant loss of copper may also be a factor resulting in the low circulating copper found.

**Summary**

1. The plasma copper and iron levels, the hematocrit and hemoglobin concentration and the total plasma protein concentration were significantly lower in patients with kwashiorkor than in controls of the same age group.
2. There was a significant diminution in total circulating copper in the kwashiorkor patients.

3. Paraphenylene diamine oxidase activity was lower in the patients with kwashiorkor than in controls, indicating that there is a diminution in the level of the active group of ceruloplasmin which contains copper.

4. Whether the low plasma copper found is due to dietary deficiency, to malabsorption of copper, or whether it is secondary to inadequate protein intake is discussed.

SUMMARIO IN INTERLINGUA

1. Le nivellos plasmatic de cupro e de ferro, le hematocrite, de concentra- tion de hemoglobina, e de concentration de proteina total in le plasma esseva significativemente reducte in patientes con kwashiorkor in comparation con subjectos de controlo in le mesme gruppo de etate.

2. Esseva constatate un diminution significative del cupro total in le circulation de patientes con kwashiorkor.

3. Le activitate de paraphenyleno-diamino-oxydase esseva plus basse in patientes con kwashiorkor que in subjectos de controlo. Isto indica un diminu- tion del nivello del gruppo active de ceruloplasmina le qual contine cupro.

4. Es discutite le question si le basse valomes pro cupro in le plasma de patientes con kwashiorkor es causate per deficientias dietari, per malabsorption de cupro, o si illo es secundari al inadegiuatia del ingestion de proteina.

REFERENCES


Plasma Copper and Iron Levels and Plasma Paraphenylenediamine Oxidase Activity (Plasma Copper Oxidase Activity) in Kwashiorkor

B. REIFF and H. SCHNIEDEN

Updated information and services can be found at:
http://www.bloodjournal.org/content/14/8/967.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml