Role of Copper in Iron Localization in Developing Erythrocytes

By Joseph R. Goodman and Peter R. Dallman

Copper deficiency in the experimental animal results in a hypochromic microcytic anemia,1,2 similar to that observed in certain conditions characterized by defective hemoglobin synthesis. Thus, a role for copper in the production of hemoglobin was proposed and actively investigated by Cartwright, Wintrobe, Lee and coworkers3-7 in copper-deficient swine. Their studies indicated that copper deficiency produces a defect in the release of iron to the circulation from the intestinal mucosa, reticuloendothelial system and the hepatocyte3-5; the pathways of porphyrin and heme synthesis in the developing erythrocyte are not affected.6 However, swine receiving iron supplementation accumulated large amounts of stainable iron in erythroid precursor cells, even though they remained somewhat anemic.5 This suggested an additional defect of intracellular iron metabolism in the copper-deficient animal.

When we examined the localization of ferritin or electron-dense iron accumulations in developing erythrocytes of copper-deficient rats, ferritin was present in the vesicles of erythroid precursors but not in the mitochondria, where iron combines with protoporphyrin to form heme. Some conditions that interfere with heme or globin synthesis, e.g., lead poisoning,8 thalassemia,9 porphyria cutanea tarda,10 result in an accumulation of iron in the mitochondria. In our study, normal animals treated with lead accumulated iron within storage vesicles and in mitochondria, as reported previously.8 In contrast, copper-deficient rats treated with lead accumulated iron in the vesicles but not in the mitochondria of erythroid precursors.

Methods

Suckling 10-day-old male Wistar strain rats were provided either a copper-poor (0.35-0.5 ppm copper) regimen (B, 1 through 4, Table 1), or a complete regimen (A-1, 2—Table 1), each diet consisting of partially skimmed milk with a vitamin and mineral supplement which contained no additional iron or copper salts.11 Ferrous ammonium citrate 1.57 Gm./liter of drinking water was provided ad libitum to both regimens. The complete dietary regimen (A-1, 2) contained, in addition, 10 mg. copper/L. drinking water as copper acetate. A complete dietary regimen of a stock pellet diet (15 ppm copper) was provided to another group (A-3).

Iron dextran (Imferon) was administered intramuscularly to copper-deficient animals (B-2) starting at 33 days of age in a dose of 5 mg. elemental iron 3 times per week. 7.5

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Table 1.—Iron Accumulation in Developing Erythrocytes

<table>
<thead>
<tr>
<th></th>
<th>Number of Animals</th>
<th>Beticuloeny Iron*</th>
<th>Erythroblast Iron</th>
<th>Hgb. mean Gm./100 Ml.</th>
<th>Range Gm./100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vesicles</td>
<td>Mitochondria</td>
<td>Vesicles</td>
<td>Mitochondria</td>
<td></td>
</tr>
<tr>
<td>A. Complete diet groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Copper supplemented</td>
<td>6</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>2. Copper supplemented &amp; Pb</td>
<td>4</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>3. Stock diet &amp; Pb</td>
<td>3</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. Copper deficient groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Copper deficient</td>
<td>5</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>2. Copper deficient + Fe</td>
<td>8</td>
<td>+++</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>3. Copper deficient + Pb</td>
<td>5</td>
<td>++</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>4. Copper deficient + Fe and Pb</td>
<td>7</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* The iron present as electron dense material in each structure is graded + to ++++ scale.
0 = no iron accumulations.
+ = very rare or sparse.
mg. lead as lead acetate was given 5 times/week starting at 45 days of age to groups of control (A-2, 3) and copper-deficient animals (B-3). An additional group of copper-deficient animals received a combination of these iron and lead regimens (B-4). All animals were killed between 63 and 75 days of age. Hemoglobin was measured as cyanmethemoglobin. Bone marrow particles from the femur were prepared for electron microscopy by fixing for 70 minutes at 4°C in osmium tetroxide buffered with s-collidine, pH 7.4, dehydrated through alcohol and propylene oxide, then embedded in Maraglas. Thin sections were cut on an MT-2 Porter Blum Microtome, stained with uranyl acetate and lead citrate, and examined with an RCA EMU 3F electron microscope.

RESULTS

In rats given a copper-deficient (B) or complete dietary regimen (A), erythroblasts and reticulocytes contained small vesicles (0.09-0.12 μ) with few electron-dense particles around the periphery, which could be identified at higher magnifications as ferritin by the characteristic aggregation in hexads of electron-dense particles. Larger vesicles packed with electron-dense particles were rare (Table 1). The erythroblasts and reticulocytes of copper-deficient animals given intramuscular iron (B-2) contained increased amounts of iron in these large vesicles (0.23-0.66 μ diameter), but none in the mitochondria. After lead treatment, animals receiving a complete diet (A-2, 3) had marked accumulations of iron in the mitochondria of reticulocytes as well as in the vesicles (Fig. 1). However, in copper-deficient rats treated with lead B-3, 4), iron was not visible in the mitochondria, even though large accumulations were observed in vesicles (Fig. 2). When lead injections were maintained for an additional 5-12 days in 7 copper-deficient animals, iron accumulation was sparse and restricted to the vesicles. In copper-deficient rats which

Fig. 1.—Parts of three reticulocytes from an animal treated with lead acetate (group A-2). Electron dense material is seen in both vesicles (V) and the mitochondria (M). Scale = 1 μ.
received iron and lead regimens (B-4), accumulations of iron were visible in vesicles but not in mitochondria of reticulocytes. Animals on a complete diet treated with lead (A-3) rarely exhibited accumulations of iron in the mitochondria of the erythroblasts; copper-deficient lead-treated rats (B-3) never did.

Lead treatment or copper deficiency, or a combination, resulted in moderate anemia (Table 1) which appeared to be partly overcome by iron-loading.

A few enlarged mitochondria were seen in the erythroblasts and reticulocytes of all copper-deficient animals (B), as observed in heart muscle and liver in copper-deficient rats and liver of mice treated with copper-chelating agent. Enlarged and vacuolated mitochondria were seen occasionally in lead-injected animals (A-2, B-3, 4).

DISCUSSION

The present study indicates that copper plays a role in the intracellular accumulation of iron at the site of heme production in the mitochondria of the developing erythrocyte. Lead administration, by interfering with heme synthesis, resulted in readily detectable accumulations of iron in the mitochondria of the reticulocyte and a moderate anemia. In reticulocytes of the copper-deficient rat, iron accumulated in vesicles but not in mitochondria—even under conditions of iron loading. This is in accord with the findings of Lee et al. that copper-deficient swine treated with iron accumulated iron in developing erythrocytes but remained mildly anemic. Since no defect in porphyrin or heme synthesis has been demonstrated in copper deficiency,
the present results lend additional support to the suggestion that copper deficiency interferes with intracellular iron utilization.

Two roles might be postulated for copper in the intracellular transport of iron in the developing erythrocyte. First, there may be a defect in the release of ferritin iron from vesicles to other intracellular sites, similar to that observed from the intestinal tract, hepatocyte, and reticulo-endothelial system to the circulation. The mobilization of iron from these storage sites appears to depend upon the oxidation of iron to the ferric form, which combines with the transport protein, transferrin. The oxidation of iron appears to be enzymatically mediated by the copper-containing protein, ceruloplasmin. The transfer of iron from the storage sites to the mitochondria could require a copper-containing enzyme which fulfills a similar intracellular function. A second possible mechanism relates to the energy requirement for the entry of iron into developing-erythrocyte mitochondria. This step is dependent upon the production of high energy phosphate bonds. Since there is a marked depletion of cytochrome oxidase in many tissues of the copper-deficient rat it is possible that a deficiency of this enzyme, which plays a major role in oxidative phosphorylation, is responsible for decreased mitochondrial uptake of iron.

The differences between iron distribution in erythroblasts and reticulocytes noted in our study (Table 1) is unexplained. Bessis and Jensen described iron accumulation in the mitochondria of erythroblasts and reticulocytes in lead-treated rats. Tanaka, as we, found that mitochondrial iron accumulations were primarily in the reticulocytes, while iron-filled vesicles were present in both cell types. The contrasting iron distributions in the two cell types may reflect a greater sensitivity of the reticulocyte to lead.

Copper-deficient rats treated with lead for the longest duration had sparser accumulations of iron in the vesicles of both types of erythrocytic cells than animals treated for a shorter period. Jandl et al. in their in vitro studies showed that lead can interfere with hemoglobin synthesis by blocking heme synthesis at low concentrations and by decreasing the uptake of iron by the erythrocyte at higher concentrations. The larger cumulative doses of lead used in our study may also interfere with the uptake of iron by the cell.

**SUMMARY**

The localization of iron in developing erythrocytes of normal and copper-deficient rats was studied by electron microscopy. Lead treatment, by interfering with heme synthesis, resulted in accumulation of iron in mitochondria and vesicles of the developing erythrocyte in normal animals. In similarly treated copper-deficient rats, iron did not accumulate in the mitochondria although it was concentrated in vesicles. The copper-deficient animal, therefore, seems to have an impaired uptake of iron by the mitochondria where it combines with protoporphyrin to form heme, in addition to the previously recognized defect in the release of iron into the circulation from the intestinal mucosa, reticulo-endothelial system and hepatocyte.

**SUMMARIO IN INTERLINGUA**

Le localisation de ferro in erythrocytos in stato de disveloppamento ab rattos...
normal e ab rattos a carentía de cupro esseva studiate per microscopía electronic. Tractamento a plumbo—interferente in le synthese de hem—resultava in le accumulation de ferro in mitochondrios e vesiculos del erythrocytos in stato de disveloppamento ab animales normal. In similemente tractate rattos a carentia de cupro, ferro non se accumulava in le mitochondrios ben que illo esseva concentrata in vesiculos. Per consequente, le animal a carentia de cupro pare haber un defective acceptation de ferro del parte del mitochondrios durante su combinacon con protoporphyrina pro le formation de hem a parte le previamente recognoscite defecito in le liberation de ferro ad in le circulation ab le mucosa intestinal, le sistema reticuloendothelial, e le hepatocytos.

ACKNOWLEDGMENTS
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