Effects of iron on copper metabolism and copper on iron metabolism in rats

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Owen, Charles A., Jr. Effects of iron on copper metabolism and copper on iron metabolism in rats. Am. J. Physiol. 224(3): 514–518. 1973.—Male Sprague Dawley rats were fed whole milk powder (Cu, Fe deficient), milk powder plus Cu in the drinking water (Fe deficient), or milk powder plus iron in the drinking water (Cu deficient). Studies with 67Cu were carried out for the most part after the rats had been on the diet for at least 300 days. Blood hemoglobin decreased in all three groups; the change was fastest in the Cu, Fe-deficient rats and slowest in the Cu-deficient rats. Ceruloplasmin activity decreased to 10% of normal or lower within 3 weeks in the Cu, Fe-deficient and Cu-deficient rats. Details of organ contents of Cu and Fe in the three groups are given. Intravenously injected 67Cu was retained longer by the Cu-deficient and Cu, Fe-deficient rats. Both groups excreted little 67Cu in their bile. Isolated livers from both groups also excreted very little 67Cu, secreted more ceruloplasmin 67Cu into the blood, and retained more 67Cu in the liver itself. Fe-deficient rats treated 67Cu normally in all respects, except for a tendency toward greater hepatic uptake of the isotope. Severely copper- and iron-deficient rats transferred ferric and ferrous 59Fe equally well into the hemoglobin of circulating erythrocytes and did so more rapidly than normal, despite the low levels of plasma ceruloplasmin. Copper-deficient rats accumulated large amounts of iron in their livers.

ceruloplasmin; copper deficiency; iron deficiency; isolated rat liver

That copper is required for the utilization of iron by animals was proposed in 1928 by Hart et al. (3) and has been confirmed repeatedly. Regardless of the precise mechanism, anemia resulting from a deficiency of copper and iron cannot be corrected by administration of either metal without the other. This has led to many studies of the influence of copper on iron metabolism, but there have been few on iron’s effect on copper metabolism. The latter was explored in the present work using milk-fed rats.

METHODS AND MATERIALS

Animals. Male Sprague Dawley white rats were used. Control rats were maintained on a normal rat diet (Purina laboratory chow, Ralston Purina Co., St. Louis, Mo.). Experimental rats were started on the special diets when their weights were about 150 g. These diets were: 1) an iron- and copper-deficient diet consisting solely of dried whole-milk powder (Mimi whole-milk powder, Maple Island Inc., Stillwater, Minn.) plus iron- and copper-free glass-distilled water (Cu, Fe deficient rats); 2) a copper-deficient diet consisting of milk powder and distilled water containing 900 mg of ferrous ammonium sulfate per liter (Cu-deficient rats); and 3) an iron-deficient diet consisting of milk powder plus distilled water containing 20 mg of cupric acetate per liter (Fe deficient).

Intakes of copper and iron were calculated from the average consumption of food and water by normal and diet-fed rats, weighing about 300 g, kept in metabolic cages until their weight remained constant for 3 consecutive days. The amounts of food and water consumed agreed with our previous observations (12) as well as those of Bellamy et al. (1).

Since the availability of copper and iron in commercial rat chow is not known, it was decided to base the supplementation of the metal salts in the drinking water on balance studies for copper and human data for iron. Thus, normal adult male rats were found to ingest and excrete 200–300 μg Cu/day. Since fecal excretion contained at most 12 μg biliary Cu/day (0.48 μg/hr ± 0.03 sp), absorption is probably about this amount. Copper supplementation was set at approximately 10 times as much: 138 μg/day (134 μg in water and 4 μg in food). Iron supplementation for the copper-deficient rats was 524 μg/day, or about what adult human beings absorb, although far below the iron contained in normal rat chow (8.8 mg/day in our studies and 15 mg/day reported by Cuthbertson (2)). Copper and iron intakes, respectively, by the Cu, Fe-deficient rats averaged 4 and 39 μg/day; the Cu-deficient rats ingested 3.7 μg Cu/day, and the Fe-deficient rats, 37 μg Fe/day.

Except where specified, experimental rats had been on their diet at least 200 days, and usually more than 300 days, when studied. Hematocrit values were: normal rats, 44.0 ± 2.4% (mean ± sp); Cu, Fe deficient, usually <20%; Cu deficient, <30%; and Fe deficient, <25%. Blood hemoglobin values were: normal, 14.3 ± 1.28 g/100 ml (mean ± sp); Cu, Fe deficient, <6 g/100 ml; Cu deficient, <10 g/100 ml; and Fe deficient, <8 g/100 ml. A detailed account of the copper concentration in various organs of such Cu, Fe-deficient rats has been reported (15).

Radiocopper. 67Cu as cupric chloride was obtained in the carrier-free state from the US Atomic Energy Commission, Oak Ridge, Tenn. It was diluted with copper-free isotonic saline before use. After intravenous injection into the rats, residual whole-body 67Cu was measured in a special small-animal whole-body counter (21).

In some rats, biliary fistulas were created about 15 hr
before the intravenous administration of the radiocopper; after the injection, bile was collected for 5 hr (13).

Isolated livers were perfused with pooled, normal, heparinized rat blood to which $^{67}$Cu was added (16, 20). Every hour, biliary $^{67}$Cu, blood $^{67}$Cu, and plasma $^{67}$Cu were assayed. Radioceruloplasmin was measured as the plasmatc $^{67}$Cu not bound to added diethyldithiocarbamate (DTC) (14), the DTC having been removed by adsorption onto activated carbon powder (Norit); this ignores the trace Cu-containing oxides also present in plasma. Originally the carbon powder was removed by filtration through cotton. Subsequently it was found to be easier and more accurate to remove it by suction through a 0.20-μ filter, onto the center of which a minute drop of acetyl alcohol had been touched with a stirring rod. (This effectively minimized foaming during filtration.)

Stable copper and iron were measured by atomic absorption spectrometry (Perkin-Elmer 303). Plasma was diluted at least twofold with water. Organs were digested with hydrochloric and perchloric acids. Organ concentrations of iron and copper were corrected for these metals present in the blood within the organs, as previously described (15). Bone marrow copper and iron were calculated by analyzing for these elements in paired femurs with epiphyses removed; the marrow was forcibly removed from one femur with jets of Cu- and Fe-free water. After the latter femur was split, it was blotted gently.

Ceruloplasmin in plasma was measured by the rate of oxidation of p-phenylenediamine at room temperature. Its activity is expressed in arbitrary units (1,000 × absorbance change ml$^{-1}$ min$^{-1}$) (18).

Hematologic changes. $^{59}$Fe as ferric chloride or ferrous citrate was injected intravenously over a 15-min period into normal and deficient rats. Small samples of blood were collected frequently over a 3- to 4-day period, and $^{59}$Fe in the saline-washed erythrocytes was measured. Normal rats converted about 60% of the $^{59}$Fe into erythrocytic hemoglobin much faster than normal.

### RESULTS

#### Hematologic changes. The progressive hematologic changes in the various groups of rats are listed in Table 1. Anemia developed most rapidly in the Cu,Fe-deficient rats and least rapidly in the Cu-deficient ones. Ceruloplasminic activity diminished rapidly in rats lacking copper, whether iron was also deficient or not. This rapid decrease was noted previously in doubly deficient rats (17).

#### Tissue Cu and Fe. In Table 2 are shown the iron and copper concentrations of various organs of the rats. Supplementation of the milk powder diet with copper (Fe-deficient) maintained an approximately normal level of copper in most organs and a level considerably above normal in the liver and kidney. Iron concentrations in these Fe-deficient rats were approximately the same as in the doubly deficient rats.

When the milk powder diet was supplemented with iron (Cu-deficient), the tissue concentrations of iron were variable. Significant increases occurred in the liver and testis. Slight increases were found in kidney and skeletal muscle. On the other hand, there was a marked depletion in the marrow and large bowel and more modest decreases in erythrocytes and plasma. In all other organs, iron content remained normal. The concentrations of copper were as low as in the Cu,Fe-deficient rats. The concentrations of copper and iron in the liver, kidney, spleen, and heart are close to those reported by Marston's group (5), despite the differences in type of rat, diet, and duration of dietary regimen.

**Whole-body retention of $^{67}$Cu.** When cuprie$^{67}$Cu chloride was given intravenously, two Fe-deficient rats retained the isotope normally and two Cu-deficient ones lost the radioisotope at a decreased rate, as in Cu,Fe-deficient rats (Fig. 1).

**Biliary excretion of $^{67}$Cu by whole rat.** A prominent distinction between the normal rat and one severely Cu, Fe-deficient is the very limited biliary excretion of copper and $^{67}$Cu by the latter (17). As shown in Table 3, in 5 hr normal rats excreted 6.5% of the intravenously administered dose of $^{67}$Cu (SD = 13.90) and Cu,Fe-deficient rats excreted less than 0.2% of the dose. The Cu-deficient rat resembled the doubly deficient rats; the Fe-deficient rats excreted radio-copper normally.

**Isolated liver perfusions.** Cu, Fe-deficient livers had twice the normal hepatic uptake of $^{65}$Cu, slightly increased secretion of radioceruloplasmin into the plasma, and almost no biliary excretion of the isotope (Table 4). Cu-deficient livers functioned like Cu,Fe-deficient ones; Fe-deficient livers secreted and excreted $^{67}$Cu normally.

**Hematopoiesis.** The rate at which $^{59}$Fe in ferrous and ferric states was incorporated into circulating hemoglobin after slow intravenous injection was measured in several Cu, Fe-deficient and Fe-deficient rats and compared with incorporation in normal rats. No clear distinctions could be found between the rates of incorporation of the two radioisrons or of the absolute amount of incorporation. Both groups of rats with both radioisrons incorporated the $^{59}$Fe into hemoglobin much faster than normal.

### Table 1. Sequential blood hemoglobin and ceruloplasmin (PPD oxidase activity) in rats on deficient diets

<table>
<thead>
<tr>
<th>Days on Diet</th>
<th>Cu, Fe Deficient</th>
<th>Fe Deficient</th>
<th>Cu Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb, g/100 ml</td>
<td>PPD, U/ml</td>
<td>Hb, g/100 ml</td>
</tr>
<tr>
<td>0</td>
<td>13.97</td>
<td>*</td>
<td>13.90</td>
</tr>
<tr>
<td>8</td>
<td>14.55</td>
<td>51.8</td>
<td>15.15</td>
</tr>
<tr>
<td>14</td>
<td>13.91</td>
<td>16.8</td>
<td>14.30</td>
</tr>
<tr>
<td>21</td>
<td>14.27</td>
<td>10.1</td>
<td>13.68</td>
</tr>
<tr>
<td>28</td>
<td>12.68</td>
<td>7.2</td>
<td>13.48</td>
</tr>
<tr>
<td>46</td>
<td>9.48</td>
<td>6.7</td>
<td>9.39</td>
</tr>
<tr>
<td>57</td>
<td>9.22</td>
<td>9.2</td>
<td>9.66</td>
</tr>
<tr>
<td>74</td>
<td>7.50</td>
<td>4.1</td>
<td>10.32</td>
</tr>
<tr>
<td>90</td>
<td>5.48</td>
<td>3.7</td>
<td>9.42</td>
</tr>
<tr>
<td>123-150</td>
<td>5.04</td>
<td>3.7</td>
<td>6.95</td>
</tr>
<tr>
<td>187-200</td>
<td>3.82</td>
<td>3.3</td>
<td>6.15</td>
</tr>
</tbody>
</table>

There were eight rats in each group; two were tested at each interval and the mean values are listed. * Normal in our laboratory, 34.1 U/ml (sd, ± 10.2) (18).
TABLE 2. Copper and iron contents of tissues of normal rats and rats on deficient diets more than 200 days

<table>
<thead>
<tr>
<th>Type of Rat</th>
<th>Copper*</th>
<th>Iron</th>
<th>Copper*</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, n = 20</td>
<td>1.24 ± 0.30</td>
<td>446 ± 44</td>
<td>0.167 ± 0.064</td>
<td>117 ± 34</td>
</tr>
<tr>
<td>Cu,Fe Deficient, n = 6</td>
<td>2.85 ± 0.52</td>
<td>17.6 ± 3.41</td>
<td>2.59 ± 0.42</td>
<td>11.5 ± 1.2</td>
</tr>
<tr>
<td>Fe Deficient, n = 3</td>
<td>3.13 ± 1.12</td>
<td>32.7 ± 4.91</td>
<td>2.77 ± 0.192</td>
<td>11.9 ± 1.72</td>
</tr>
<tr>
<td>Cu Deficient, n = 4</td>
<td>1.95 ± 0.50</td>
<td>160 ± 27</td>
<td>3.62 ± 0.47</td>
<td>10.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>3.73 ± 0.30</td>
<td>13.3 ± 2.7</td>
<td>3.14 ± 0.62</td>
<td>14.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>0.106 ± 0.012</td>
<td>291 ± 64</td>
<td>2.76 ± 0.68</td>
<td>17.8 ± 1.8</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Recognition of the body's need for both copper and iron to prevent anemia dates back to 1928 (3). Although the mechanism is still imperfectly understood, it appears that iron is absorbed and stored adequately, whether copper is normal or deficient in the diet, but only if there is sufficient copper can the stored iron be released to enter into hemopoiesis. If copper is deficient, the animal stores iron excessively (5) but releases it when copper is replenished. The releasing agent is the copper carrier, ceruloplasmin, because this protein, but not copper salts, induces release of iron from iron-laden livers in perfused liver systems (9, 10). How ceruloplasmin releases stored iron is uncertain, although ceruloplasmin's ability to oxidize ferrous iron ("ferroxidase") has been proposed (6-9, 11).

The current study confirmed the exaggerated hepatic uptake of iron when the diet was deficient in copper. It
further showed that anemia developed most rapidly when both metals were deficient in the diet [hemoglobin fell by half in 2.5 months, approximately the life-span of the rat erythrocyte (19)], as opposed to iron deficiency alone (5 months) or copper deficiency alone (10 months). A detailed account of tissue copper and iron in the three deficiency states has not been presented before. In essence, excess hepatic storage of iron in copper deficiency must depend on adequate intake of iron and is not simply a relocation of body iron.

The iron levels tended to be normal in Cu-deficient rats, except in the liver, muscle, and testis, where they were increased, and in the blood and marrow, where they were decreased. The decrease in iron in the marrow is compatible with ineffective release of iron stored elsewhere, in turn leading to anemia. The normal content of iron in the muscle and testis is not explained.

Since copper is known to be required for normal utilization of iron, which is fully confirmed by the present study, one might suspect that the reverse could also be true, that is, the storage of copper might be increased and synthesis of ceruloplasmin diminished in iron deficiency. There was a tendency toward increased uptake of 67Cu and decreased release of ceruloplasmin 67Cu in 5 hr perfusions of isolated livers from rats that had been on the Fe-deficient diet less than 6 months. However, more prolonged iron deficiency had no clear effect on copper metabolism, and rats on a diet deficient in copper and iron were virtually indistinguishable from comparable rats with dietary iron supplements. Plasma ceruloplasmin decreased 80% within 3 weeks in both Cu-deficient and Cu, Fe-deficient rats. Both types of rats retained 67Cu considerably longer than normal, and they excreted very little 67Cu in their bile.

The Cu, Fe-deficient rats incorporated both ferric and ferrous 57Fe into circulating erythrocytes at least as efficiently as normal, despite plasma ceruloplasmin levels of less than 10% of normal. Either this is sufficient ceruloplasmin or perhaps nonceruloplasminic ferroxidases can accomplish the oxidation of iron necessary for its combining with apotransferrin (4, 22, 24). The most significant iron abnormality in Cu-deficient rats was the marked increase in hepatic iron, which conforms to the concept of the need for ceruloplasmin to release iron stores.

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REFERENCES

14. Owen, C. A., Jr. Metabolism of radiocopper (Cu
16. Owen, C. A., Jr., and J. B. Hazelrig. Metabolism of 