THE RELATION OF COPPER TO CYTOCHROME OXIDASE AND HEMATOPOIETIC ACTIVITY OF THE BONE MARROW OF RATS

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Recent studies have shown that copper deficiency of the rat leads to a great reduction of the cytochrome oxidase activity of the liver and the heart (1). Considering the fact that in extra-uterine life the bone marrow is the main center of hematopoietic activity and that copper is necessary for normal hematopoiesis, it was of interest to determine cytochrome oxidase activity of the bone marrow (a) during copper deficiency and recovery from it, and (b) in other conditions of diminished or accelerated hematopoietic activity. The results of such a study are reported here.

EXPERIMENTAL

Animals—Iron deficiency and copper deficiency in rats were produced as described previously (1). The milk, after H₂S treatment and concentration in vacuo, was fortified with sufficient thiamine chloride to insure a daily intake of at least 20 \( \mu \) of this vitamin. All metallic supplements were fed with a small quantity of milk. The rats in Groups 10 to 13 received the stock diet of Purina Dog Chow. Group 11 was subjected to severe hemorrhage by heart puncture under light ether anesthesia by which 20 to 30 per cent of the assumed blood volume (7 per cent of the body weight) was removed.

The animals in Group 12 were exposed to low oxygen tension by keeping them in pairs in a large desiccator evacuated to 420
to 430 mm. of Hg. A constant flow of air was drawn through the desiccator and the vacuum was regulated by a capillary stop-cock.

Cytochrome Oxidase Determination—The rats were decapitated and the long bones (humeri, femora, tibiae) were dissected. The marrow was removed from the bones after they were split longitudinally with a sharp scalpel. It was then suspended in redistilled water and homogenized (2). An aliquot of the marrow suspension was dried to constant weight at 105° and the remainder was used for determination of cytochrome oxidase activity. This determination was carried out manometrically as described earlier (1). Each of two duplicate Warburg vessels contained 0.75 ml. of marrow suspension, 0.1 ml. of cytochrome c (1 × 10⁻⁷ M), 0.2 ml. of neutral semicarbazide (2.8 × 10⁻⁶ M), and 1.5 ml. of m/15 phosphate buffer, pH 7.2. The side arm contained 0.25 ml. of hydroquinone (5 × 10⁻⁶ M) and the center cup contained 0.2 ml. of 20 per cent KOH. A control vessel without added cytochrome c was always run in parallel. The determinations were made at 37.2° and for a 25 minute period after equilibration and closing of the stop-cocks. The cytochrome oxidase activity was calculated from the oxygen uptake of the system (minus the blank) during a 20 minute interval (between the 5 and 25 minute periods) and expressed as $Q_{\text{oxidase}} = \frac{\text{e.mm. of oxygen absorbed}}{\text{hour} \times \text{mg. of dry marrow}}$.

DISCUSSION

Table I summarizes the results. The bone marrow of young rats in full hematopoietic activity has an oxidase quotient of 30 to 35 (Group 1). Mature rats which need to make erythroid elements only for maintenance of the normal blood picture have a distinctly lower cytochrome oxidase activity of the bone marrow (Groups 2 and 10). In severe anemia of iron deficiency the cytochrome oxidase activity of the bone marrow corresponds to that found in rats with full hematopoietic activity (Group 3). It appears that under these conditions the bone marrow is prepared for hematopoietic activity and that it lacks only iron for incorporation into the hemoglobin molecule and perhaps for the synthesis of other heme compounds. In copper deficiency, however, the cytochrome oxidase activity of the bone marrow is very low.
In this case the hematopoietic failure is associated with an enzyme deficiency in the bone marrow. This is emphasized by the fact that in early copper deficiency, when the development of anemia is accelerated as a result of rapid growth and increasing blood volume, the bone marrow is already deficient in cytochrome oxidase activity (Group 5). Most striking, however, is the immediate response of the copper-deficient bone marrow to copper therapy. 24 hours after 0.1 mg. of copper was fed to severely deficient rats, the bone marrow had attained a cytochrome oxidase activity similar to that found in marrows with

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Diet</th>
<th>Condition of rats and treatment</th>
<th>No. of rats</th>
<th>Hb</th>
<th>Q oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk + Fe + Cu + Mn</td>
<td>Young, growing</td>
<td>10 0-12</td>
<td>33.4 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Same</td>
<td>Mature</td>
<td>8 12-14</td>
<td>19.8 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Milk + Cu + Mn</td>
<td>Fe-deficient</td>
<td>15 3-5</td>
<td>31.9 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Milk + Fe + Cu-deficient</td>
<td>21 2-4</td>
<td>5.6 ± 3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Same</td>
<td>Early Cu deficiency; 0-2 wks. after weaning</td>
<td>10 4-6</td>
<td>10.4 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>Cu-deficient, fed 0.1 mg. Cu 24 hrs. previously</td>
<td>11 2-4</td>
<td>29.0 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>Cu-deficient, fed 0.1 mg. Cu per day 2 days</td>
<td>10 3-4</td>
<td>29.6 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>Cu-deficient, fed 0.1 mg. Cu per day 5 days</td>
<td>10 5-6</td>
<td>34.2 ± 7.0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td>Cu-deficient, fed 2 mg. Co during previous 3½ days</td>
<td>10 2-4</td>
<td>6.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Stock diet</td>
<td>Mature</td>
<td>12 12-14</td>
<td>18.6 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Same</td>
<td>&quot; hemorrhage 48 hrs. previously</td>
<td>10 8-10</td>
<td>34.1 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>Mature; kept under low O2 tension 42 hrs. previously</td>
<td>10 12-14</td>
<td>31.5 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td>Mature; fed 2 mg. Co during previous 3½ days</td>
<td>10 12-14</td>
<td>29.7 ± 3.7</td>
<td></td>
</tr>
</tbody>
</table>
full hematopoietic activity (Group 6). This response is practically maximal, since the cytochrome oxidase activity of the bone marrow does not increase appreciably when copper therapy is prolonged (Groups 7 and 8). It may be well to point out that 24 hours after the initial copper therapy a reticulocyte response in the circulating blood is hardly noticeable (3). The results presented here suggest that the cytochrome oxidase of the bone marrow must be brought to or maintained at a certain level (as yet quantitatively undetermined) before hematopoiesis can proceed successfully. It is clear that for this process copper must be available to the animal. The entrance of copper into the bone marrow within 24 hours following copper therapy has been demonstrated by means of radioactive copper (4). The observations reported here on the effect of copper on the cytochrome oxidase activity of the bone marrow are in harmony with the results previously found with liver and heart (1).

If cytochrome oxidase activity of the bone marrow is a prerequisite for and closely associated with hematopoietic activity, it should be possible to observe increased cytochrome oxidase activity in the bone marrow of mature, normal rats which have been subjected to hematopoietic stimuli for very short periods. Severe hemorrhage, low oxygen tension, and feeding of cobalt salts can be used as powerful stimuli for hematopoietic activity of the bone marrow. Under all three conditions there is a rapid increase of the cytochrome oxidase activity of the bone marrow to a level associated with rapid blood formation (Groups 11 to 13). Cobalt alone cannot elicit such a response, since copper-deficient rats do not respond to the stimulus of cobalt (Group 9). Underhill et al. (5) have shown that copper-deficient rats fail to develop cobalt polycythemia. The effect of copper on cytochrome oxidase activity is therefore specific. Normal mature rats contain or have access to sufficient copper to permit the formation of increased cytochrome oxidase activity in the bone marrow under the influence of hematopoietic stimuli.

The observations reported here demonstrate clearly that (1) a high cytochrome oxidase activity of the bone marrow is intimately associated with hematopoiesis, provided the latter process is not interfered with by other deficiencies, and (2) copper is essential for the formation and the maintenance of cytochrome oxidase activity of the bone marrow.
It appears, therefore, that the effect of copper on blood formation can be accounted for, at least in part, by its ability to provide for adequate enzymatic and metabolic activity at the site of hematopoiesis. In view of the low oxygen tension in the erythrogenic centers and their great synthetic activity, such a concept does not appear unreasonable. It has been suggested that the copper content of the blood may be of functional significance in relation to blood formation (6, 7). Further work will be necessary to determine whether the copper content of the blood bears any relation to the cytochrome oxidase activity of the bone marrow.

The chemical nature of the compound (or compounds) responsible for cytochrome oxidase activity of animal tissues is not yet fully understood. A metalloporphyrin with a hemochromogen spectrum is apparently involved in the cytochrome oxidase activity of heart muscle preparations (8). It has been suggested that copper may be a structural component of cytochrome oxidase (9). If this were true, it would provide, together with the observations presented here, a rational explanation for the effect of copper on hematopoiesis. Until isolation permits an analysis of cytochrome oxidase, such reasoning remains largely speculative. If, on the other hand, cytochrome oxidase is an iron-porphyrin compound, its synthesis, like that of hemoglobin and of catalase (10), depends on the presence of copper. In that case the effect of copper on hematopoiesis would be dual, first on cytochrome oxidase activity of the bone marrow and second on the synthesis of hemoglobin.

**SUMMARY**

1. Copper deficiency causes a great decrease of the cytochrome oxidase activity of the bone marrow of rats.
2. Copper therapy of deficient rats initiates an immediate increase in cytochrome oxidase activity of the bone marrow. Within 24 hours maximum activity is approached.
3. Iron, manganese, or cobalt does not affect cytochrome oxidase activity in the absence of copper.
4. Normal mature rats respond to hematopoietic stimuli (hemorrhage, low oxygen tension, feeding of cobalt) by a rapid increase of cytochrome oxidase activity of the bone marrow.
5. There exists a close relation between the cytochrome oxidase activity of the bone marrow and its ability to form hemoglobin and erythrocytes.
BIBLIOGRAPHY

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