STUDIES ON THE GLUTATHIONE CONTENT OF THE BLOOD IN NUTRITIONAL ANEMIA*

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In a study of the copper content of the blood in nutritional anemia (1) it was observed that in pigs depleted of their bodily copper stores the copper of the blood falls to extremely low values and that upon feeding sufficient amounts of copper it is restored very rapidly to normal levels. It was suggested then that with a low copper concentration in the blood, the medium surrounding the young blood cells in the bone marrow would not possess the right physical and chemical properties to permit formation of hemoglobin and maturation of erythrocytes. Clinical observations (quoted in (1)) established that in various conditions of anemia, i.e. with increased demand for formation of hemoglobin and of erythrocytes, the copper content of the blood increases. Under similar conditions, in hemorrhagic or in phenylhydrazine anemia of rabbits (2–6), and in various forms of human anemia (2, 7, 8), the glutathione content of the erythrocytes was found to be increased. Other considerations pointed also to a possible correlation between the copper content of the blood and the level of reduced and oxidized glutathione in the blood. Tompsett (9) suggested that part of the copper inside the red blood cells may occur as the cuprous mercaptide of glutathione. The oxidation of reduced glutathione is a metallic catalysis (10), probably

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brought about by iron and copper complexes in the blood (11, 12). With these facts in mind we studied the glutathione content of the blood of rats and of pigs under conditions of retarded and accelerated activity of the hematogenic organs.

EXPERIMENTAL

The rats used in this work were made anemic as usual on an exclusive milk diet. When the hemoglobin content of the blood had decreased to 3 to 4 gm. per 100 cc., some of the animals were used for glutathione analyses; others were fed a daily supplement of 0.5 mg. of Fe, or 0.1 mg. of Cu, or 0.5 mg. of Fe + 0.1 mg. of Cu for a period of 5 days. At the end of this period, i.e. at a time when the rat shows a maximum reticulocyte response if both iron and copper are fed (13), the blood was analyzed for glutathione. The blood was withdrawn from the abdominal aorta by the procedure of Swanson and Smith (14). It was discharged into a weighed test-tube containing 5 cc. of redistilled water with oxalate as anticoagulant. After weighing, sufficient water was added to bring the proportion of blood to water to 1:8. Glutathione was determined by the method of Woodward and Fry

### Table I

**Glutathione in Rat Blood**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Hb per 100 cc.</th>
<th>Reduced glutathione</th>
<th>Total glutathione</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before gm.</td>
<td>After gm.</td>
<td>mg. per 100 gm. r.b.c.*</td>
</tr>
<tr>
<td>Stock rats, mature...................</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; 6 wks. old......................</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemic ............................</td>
<td>4</td>
<td>5.50</td>
<td>4.11</td>
<td>40.43</td>
</tr>
<tr>
<td>&quot; .................................</td>
<td>7</td>
<td>4.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 mg. Fe for 5 days..............</td>
<td>7</td>
<td>3.41</td>
<td>3.59</td>
<td>5.13</td>
</tr>
<tr>
<td>0.1 &quot; Cu &quot; 5 &quot; ..................</td>
<td>7</td>
<td>2.95</td>
<td>3.84</td>
<td>6.28</td>
</tr>
<tr>
<td>0.5 &quot; Fe + 0.1 mg. Cu for 5 days...</td>
<td>7</td>
<td>3.17</td>
<td>7.83</td>
<td>28.69</td>
</tr>
</tbody>
</table>

* Mg. glutathione in 100 gm. blood

Millions red blood cells in 1 c.mm. blood
Young, anemic rats usually do not yield enough blood to permit satisfactory analyses of both reduced and total glutathione on the same sample. The values reported in Table I were obtained from different rats.

The glutathione analyses of pig blood were made by the same method as in our studies on the copper content of the blood of anemic pigs (Pigs 20 to 27, (1)).

RESULTS AND DISCUSSION

Because the glutathione of the blood is carried only in the erythrocytes (2, 16–18), the results are expressed in terms of glutathione per million erythrocytes. In the case of rat blood we also report mg. of glutathione per 100 cc. of blood.

Table I summarizes the results obtained with rat blood. It is evident that with development of nutritional anemia the reduced glutathione content of the red cells fell to low levels. At this point it was not raised by feeding either iron alone or copper alone. But when both iron and copper were fed, i.e. during rapid recovery from nutritional anemia, there was a rapid rise of the reduced glutathione content of the erythrocytes to normal values. Quite unexpected was the observation that in nutritional anemia of rats there is a marked shift in the proportion of reduced to oxidized glutathione. Normally the reduced form accounts for 90 to 100 per cent of the total glutathione of the erythrocytes; in nutritional anemia of rats it is less than 50 per cent. Feeding iron alone tended further to depress the amount of reduced glutathione in the erythrocytes. Upon feeding both iron and copper, however, the equilibrium is apparently rapidly shifted back to the normal proportions. Three rats getting both iron and copper yielded enough blood to permit analysis for both forms of glutathione, the reduced form accounting for from 86 to 90 per cent of the total. The present knowledge of blood chemistry cannot explain the significance of these observations. It is a matter of conjecture whether the increase of total glutathione in the red cells and the shift from the oxidized to the reduced form after feeding of both iron and copper is a direct effect of the two metals on glutathione or whether it is secondary to rapid formation of hemoglobin and erythrocytes. Litarczek et al. (19) have suggested that the increased glutathione content of the erythro-

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cytes following hemorrhage in rabbits is due to the increased number of reticulocytes which contain more glutathione than the mature cells. This would tend to correlate hematogenic activity of the bone marrow with the glutathione content of the red cells. It might be argued that increased glutathione content is due to the high reticulocyte count; however, in normal animals with a low number of reticulocytes, the glutathione content of the blood is the same.

Table II
Average Glutathione Content of Pig Blood at Various Stages of Nutritional Anemia and Recovery

The results are expressed as (mg. of glutathione in 100 cc. of blood)/(millions of red blood cells in 1 c.mm. of blood).

<table>
<thead>
<tr>
<th>Hb per 100 cc. blood</th>
<th>Milk; no metals</th>
<th>Milk + Fe</th>
<th>Milk + Fe + Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reduced</td>
<td>Total</td>
<td>Per cent oxidized</td>
</tr>
<tr>
<td>gm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10–12</td>
<td>4.35</td>
<td>4.73</td>
<td>8.0</td>
</tr>
<tr>
<td>9–10</td>
<td>3.76</td>
<td>4.13</td>
<td>8.9</td>
</tr>
<tr>
<td>8–9</td>
<td>3.64</td>
<td>4.64</td>
<td>23.7</td>
</tr>
<tr>
<td>7–8</td>
<td>4.76</td>
<td>5.85</td>
<td>18.6</td>
</tr>
<tr>
<td>6–7</td>
<td>3.74</td>
<td>4.33</td>
<td>13.6</td>
</tr>
<tr>
<td>5–6</td>
<td>4.86</td>
<td>5.22</td>
<td>16.3</td>
</tr>
<tr>
<td>4–5</td>
<td>5.62</td>
<td>6.51</td>
<td>13.6</td>
</tr>
<tr>
<td>3–4</td>
<td>6.42</td>
<td>6.92</td>
<td>7.2</td>
</tr>
<tr>
<td>2–3</td>
<td>7.20</td>
<td>8.25</td>
<td>12.7</td>
</tr>
</tbody>
</table>

The results obtained with pig blood, summarized in Table II, are entirely different. Although we observed considerable fluctuation in the glutathione content of the erythrocytes in successive bleedings, it is evident that in pigs the glutathione content of the cells increased with progressive anemia and that it returned to lower values during recovery. No constant shift in the proportion of the two forms of glutathione could be observed. This is directly opposite to the results obtained with rat blood, but it conforms with the observations recorded in the literature to which we referred. It appears that in pig blood the glutathione content of the erythrocytes is dependent primarily upon the degree of
anemia of the animals. It was roughly the same in animals having about the same hemoglobin content of the blood, irrespective of their dietary intake of iron and of copper.

Iodometric titrations of blood and of tissue filtrates are not specific for glutathione. Substances known to reduce iodine besides glutathione are ascorbic acid, cysteine, and thioneine. Ascorbic acid is present in the blood at least partly in the oxidized form (20) and would therefore not interfere with the determination of GSH. It has also been shown that the ascorbic acid content of the blood depends upon dietary intake (21), which would be not very high on an exclusive milk diet. The same applies to thioneine because Potter and Franke (22) have found that the thioneine content of the blood of rats on a diet containing 20 per cent commercial, crude casein and 10 per cent butter fat was less than 0.1 mg. per 100 cc. of blood. Particularly important is the observation of Quensel and Wachholder (23) that upon destruction by formaldehyde of GSH in blood filtrates according to Woodward and Fry, the reducing value of the filtrate is lost. This conforms with the work of Woodward (24) who showed that in the determination of glutathione by glyoxalase activation, thioneine, cysteine, and ascorbic acid are not included and that the reduced glutathione determined in this manner agrees with the values obtained by iodine titration.

We cannot offer any explanation for the observed differences in the two species. It must be borne in mind, however, that although the reducing value toward iodine of blood filtrates is apparently due mostly to glutathione, the possibility exists that during anemia of pigs and perhaps other animals, reducing substances other than glutathione may appear in the blood and thus account for the increased iodine titrations.

SUMMARY

1. In rats suffering from nutritional anemia the total glutathione content of the erythrocytes is decreased.

2. At the same time there is a marked shift towards the oxidized form of glutathione, causing a great decrease in the amount of reduced glutathione in the erythrocytes.

3. During recovery from anemia as a result of feeding of iron and copper the normal conditions are rapidly restored.
4. In nutritional anemia of pigs there is an increase of both the total and the reduced glutathione content of the erythrocytes as the anemia becomes severe.

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