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

BY M. O. SCHULTZE, C. A. ELVEHJEM, AND E. B. HART

(From the Department of Agricultural Chemistry, University of Wisconsin, Madison)

(Received for publication, July 31, 1936)

The occurrence of copper in the blood of man and various animals has been known for a long time but until recently most of the analyses for blood copper were made incidental to other studies. The recorded observations on this subject have been summarized in the papers by Locke et al. (1), Sarata (2), Roncato and Bassani (3), and Tompsett (4). With the discovery of the essential nature of copper for hemoglobin formation (5) the analysis of animal organs and tissues for copper has assumed new significance and during the last few years attempts have been made to study systematically the copper content of the blood of man and animals in normal and pathological conditions. Several observations are recorded in the literature about the changes of the copper content of the blood in various conditions of anemia (1, 6–11), but, as far as we know, no data have been published on the changes in the copper content of the blood of animals suffering from nutritional anemia under well controlled conditions. Because in recovery from severe nutritional anemia the bone marrow is in a state of maximum activity, this condition should be unusually favorable for studying the copper content of the blood during rapid hematopoiesis.

Our studies on the copper content of tissues and organs of anemic pigs provided excellent material for copper analyses of the blood and made it possible to correlate the results obtained from the analyses of the various organs and tissues.

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

This work was supported in part by a grant from the Research Funds of the University.
EXPERIMENTAL

In the preceding paper (12) the methods for handling the animals, for blood collection, and for copper analysis are described. The results in Table II of that paper indicate the good agreement between the copper content of the blood obtained by direct analysis and that calculated from the copper content of the plasma, the blood cells, and from the cell volume. The blood was collected in flasks containing purified potassium oxalate as anticoagulant. Before analysis the blood cells were washed twice with purified physiological salt solution.

The copper analyses reported in Table I were obtained from the animals used in the study reported in the preceding paper (Pigs 1 to 18). The results made it desirable to follow the copper content of the blood of pigs as they developed anemia and recovered from it under the influence of iron and copper feeding. For this purpose a litter of pigs was raised and fed as in the other experiment except that small amounts of pure iron were fed to some of the animals for short periods of time in order to delay the onset of anemia. This was desirable because our previous work had indicated that pigs, particularly those born in early spring, will become severely anemic owing to iron deficiency before their bodily stores of copper are exhausted. When the animals were 5 weeks old, weekly bleedings for copper analysis were started. The blood was withdrawn under light ether anesthesia by heart puncture. The amount of blood withdrawn depended upon the expected copper content of the blood as indicated by the previous analysis. With few exceptions enough blood was withdrawn to permit copper analysis of the whole blood and of the plasma. From these values and the cell volume the approximate copper content of the blood cells could be calculated.

DISCUSSION

The results obtained from Pigs 1 to 18 are summarized in Table I. The copper content of the blood obtained at a slaughterhouse from mature hogs gave values of the same magnitude as those reported by McFarlane (13), 103 to 180 micrograms of Cu per 100 cc. of blood, and by Tompsett (4) who found 165 to 185 micrograms of Cu per 100 cc. of blood and 161 to 200 micro-
grams of Cu per 100 cc. of plasma. In the case of two pigs used as control animals for these experiments, i.e. those on a milk diet fortified with iron and copper, the copper content of the whole blood was 108 and 133 micrograms per 100 cc.; that of the plasma 135 and 138 micrograms per 100 cc. Two control pigs subjected to weekly bleedings had the following copper distribution at the
time of the last bleeding:

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Treatment; daily supplement</th>
<th>Hb (gm. per 100 cc.)</th>
<th>Cu in 100 cc.</th>
<th>Cu in r.b.c. from 100 cc. blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hb</td>
<td>Plasma</td>
<td>Blood</td>
</tr>
<tr>
<td>14</td>
<td>Anemic; no metals</td>
<td>2.50</td>
<td>8.1</td>
<td>7.8</td>
</tr>
<tr>
<td>17</td>
<td>Same</td>
<td>2.40</td>
<td>6</td>
<td>7.3</td>
</tr>
<tr>
<td>3</td>
<td>25 mg. Fe 14 days</td>
<td>3.94</td>
<td>17.43</td>
<td>98.5</td>
</tr>
<tr>
<td>7</td>
<td>Same</td>
<td>2.55</td>
<td>9.75</td>
<td>79.8</td>
</tr>
<tr>
<td>11</td>
<td>25 mg. Fe 8 days</td>
<td>2</td>
<td>7.29</td>
<td>11.1</td>
</tr>
<tr>
<td>13</td>
<td>25 &quot; &quot; 9 &quot; &quot;</td>
<td>&lt;2</td>
<td>8.10</td>
<td>9.7</td>
</tr>
<tr>
<td>5</td>
<td>2 &quot; Cu 7 &quot; 4 mg. Cu 7 more days</td>
<td>2.87</td>
<td>11.05</td>
<td>206</td>
</tr>
<tr>
<td>6</td>
<td>25 mg. Fe + 2 mg. Cu 7 days</td>
<td>5.40</td>
<td>17.35</td>
<td>231.0</td>
</tr>
<tr>
<td>8</td>
<td>25 mg. Fe + 2 mg. Cu 9 days</td>
<td>4.85</td>
<td>17.30</td>
<td>224.5</td>
</tr>
<tr>
<td>15</td>
<td>Same</td>
<td>4.51</td>
<td>14.50</td>
<td>120.3</td>
</tr>
<tr>
<td>12</td>
<td>25 mg. Fe + 2 mg. Cu 4 wks.</td>
<td>9.95</td>
<td>30.00</td>
<td>108.3</td>
</tr>
<tr>
<td>18</td>
<td>25 mg. Fe + 2 mg. Cu 24 days</td>
<td>10.11</td>
<td>28.3</td>
<td>133.0</td>
</tr>
<tr>
<td></td>
<td>Mature hogs from Madison slaughter-house</td>
<td></td>
<td></td>
<td>154.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>166.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>163.0</td>
</tr>
</tbody>
</table>

In severe nutritional anemia, however, the copper may almost disappear from the blood, the observed values being 7 and 8 micrograms per 100 cc. of whole blood and less than 4 micrograms per 100 cc. of plasma. It must be pointed out that these results
were obtained with animals suffering from both iron and copper deficiency (Pigs 14 and 17), whose livers were extremely depleted of copper. Not in all cases of severe nutritional anemia may such low values for the copper content of the blood be encountered. It was pointed out before that pigs may become severely anemic from iron deficiency alone. In that case the copper content of the blood, although reduced, does not reach the low values just mentioned. This is indicated by the values obtained with Pigs 3 and 7 which became severely anemic but responded very slowly to treatment with pure iron alone (or did not show further decrease of Hb), as shown by the hemoglobin curves in Fig. 1 of the preceding paper. The copper content of the blood of these pigs was 99 micrograms per 100 cc. of whole blood and 59 and 46 micrograms per 100 cc. of plasma. Also Pig 19, 4 weeks old, with hemoglobin less than 2 gm., had 89 micrograms of Cu per 100 cc. of blood. These facts must be strictly considered in future studies; namely, that severe nutritional anemia is not necessarily associated with extreme depletion of the copper content of the blood. Two pigs (Nos. 11 and 13), severely anemic, failed to respond to feeding of pure iron; in fact, the hemoglobin content of their blood continued to decrease. Upon analysis their blood and the livers (12) were found to be extremely low in copper. Evidently their anemic condition was due to both iron and copper deficiency. In the absence of bodily stores of copper and with a very low copper content of the blood, they were unable to form hemoglobin and erythrocytes. Analogous to this condition, only reversed, is that in which severely anemic pigs were unable to respond to treatment with copper alone (Pigs 5, 9, 10, Fig. 1 (12)). Unfortunately blood analyses from only one of these animals were obtained. The other two died suddenly during the hot and sultry days of July, 1935. From these analyses, however, it is evident that the feeding of copper raised the copper content of the blood to values higher than normal. Similarly, high values for copper were observed in pigs recovering rapidly from severe anemia under the influence of feeding iron and copper. Under the conditions of these experiments, 2 mg. of copper were supplied to the animals daily. If any of the tissues active in blood formation were in great need for copper, it should be expected that they would withdraw from the blood the copper
absorbed from the intestine. That this, apparently, is not the case to any great extent is borne out by the copper analyses of liver, spleen, and the distal ends of ribs (12).

These observations point to an intimate correlation between the copper content of the blood and the rate of hemoglobin formation. Of the tissues and organs studied in our work, the blood was the only one where a great accumulation of copper was found when copper was fed to an animal previously depleted of copper.

This might suggest the theory that, unless the copper content of the blood is maintained at a certain minimum level, hemoglobin formation cannot take place or only at a very slow rate, even if the animal has access to available iron. To get more information on this important point weekly copper determinations of the blood of pigs were made. The results are represented in Figs. 1 to 3.

Our previous observation that in severe nutritional anemia due to iron and copper deficiency the copper content of the blood
falls to very low levels is fully substantiated. It is interesting to note that this drop in blood copper at first is very rapid, from 90 to 100 micrograms to about 30 micrograms per 100 cc. of blood. When the blood copper had reached this level, the feeding of 50 mg. of pure iron per day was started. As a result we observed a slow hemoglobin regeneration in three cases (Pigs 22, 24, 25).

One animal responded more rapidly (Pig 23) for a period of 2 weeks, during which time it was able to maintain the copper content of its blood almost constant. Then, however, the copper content of the blood fell to still lower levels and hemoglobin formation in all cases became very slow or ceased, although the animals had access to an abundance of available iron. When the copper content fell to about 10 micrograms per 100 cc. of blood,
hemoglobin formation was completely arrested. It appeared, therefore, that the level of blood copper at which hemoglobin formation is possible lies somewhere between 10 and 30 micrograms per 100 cc. of blood. By feeding small amounts of copper to the pigs that had ceased to form hemoglobin, we attempted to establish at what level of blood copper hemoglobin formation would be resumed. In two cases (Pigs 22 and 24) the feeding of small amounts of copper initiated considerable hemoglobin formation, although the copper content of the blood failed to rise above 20 micrograms per 100 cc. of blood.

When larger amounts of copper were fed (4 mg. daily) we ob-

**Fig. 3.** Hemoglobin, erythrocyte, and copper content of the blood of Pigs 23 and 25. Note the rapid increase in blood copper after feeding 4 mg. of Cu to Pig 23.
served again a tremendous increase in the copper content of the blood, together with rapid hematopoiesis. In one case (Pig 23) the copper content of the blood rose in 3½ days from less than 10 to about 100 micrograms per 100 cc. If sufficient copper is supplied, the animal evidently attempts to restore the depleted copper content of the blood at once. Thus the accumulation of copper under these conditions is much greater in the blood than in the liver (12) and rapid hematopoiesis apparently can take place only if the copper content of the blood is at a fairly high level.

Some authors have postulated that copper is a specific stimulant for erythrocyte formation (14, 15). Following experimental hemorrhage Sarata and Suzuki (11) observed an increase in the copper content of the erythrocytes of rabbits, particularly marked during the first few days following the operation. Itizyo (16) suggested that “the cuprocytes [the copper-rich cells] are replaced by ordinary ones in a relatively short time.” When we followed the copper content of the blood of our pigs during production of anemia and during recovery, the calculated copper content of the erythrocytes expressed for 1 million red blood cells per c.mm. showed a marked decrease compared to normal values. But only when large amounts of copper were fed with iron did we find the copper content of the erythrocytes increased over the values calculated for the previous bleeding. Our data can neither support nor refute the alleged production of “cuprocytes” as observed by the Japanese workers, because we worked with a different species and produced nutritional rather than hemorrhagic anemia and because we could not make copper analyses immediately upon initiation of hematopoietic activity of the bone marrow. We should like to point out, however, that it is not possible to assign to copper or to iron a specific function for either formation of erythrocytes or of hemoglobin. The two processes are interdependent and although erythrocytes may vary in their hemoglobin content, they always contain some hemoglobin. Even if hemoglobin formation proceeds only slowly, enough may be formed to permit production of large numbers of cells with a low color index. We have observed this in one of our animals (Pig 22) to which only a small amount of copper was fed.

Our observation that in nutritional anemia due to iron and
copper deficiency the copper content of the blood is so much decreased is not opposed to clinical observations that the copper content of the blood is increased in various conditions of anemia (1, 6–11) and in pregnancy (17, 18). With greater demand for formation of hemoglobin and erythrocytes the organism apparently has the ability to raise the copper content of the blood from bodily stores of copper in an effort to speed up hematopoiesis. If in addition to copper other factors such as iron and the pernicious anemia factor are available, the anemia will be overcome rapidly. If one or more of these factors is absent, hemoglobin and erythrocyte formation will not take place in spite of the high copper content of the blood and anemia will persist. What controls the regulation of the copper content of the blood is unknown. Whatever that mechanism may be, it exerts its effect rapidly (11). The observation has been repeatedly made that blood serum from animals subjected to low oxygen tension contains substances which stimulate hematopoiesis (hemopoietins) (19). This effect might perhaps be correlated with an increased copper content of the serum. Somogyi (20) observed that the stimulating effect of "regeneration serum" may be further enhanced by increasing the copper content of this serum.

From the present knowledge of the factors instrumental in hematopoiesis it is difficult to appreciate the full significance of our observations. The great increase of copper in the blood of our anemic animals following copper feeding is not only the result of increased copper absorption from the intestine. It is a well known fact that the increase in the amino acid, fat, and sugar content of the blood during the absorptive period is relatively small and transient. Increased calcium intake does not lead to a permanently increased calcium level in the blood. It might be argued that our observations are of no functional significance but that the changes in the copper content of the blood are merely secondary to and concurrent with copper storage in various organs and tissues. This, however, is unlikely, because no appreciable accumulation of copper was observed in the liver and the bone marrow (12) at a time when the copper content of the blood was high and hematopoiesis rapid. It might be suggested, however, that the developing erythrocytes in which hemoglobin is formed are bathed in and nourished by the blood. Hemoglobin
formation in and maturation of the blood cells can take place only if the surrounding environment, the blood, possesses the proper physical and chemical properties. Among such properties could be conceivably oxidation-reduction potential, sulfhydryl groups, and enzymatic activation. With low copper concentration in the blood the medium surrounding the young blood cells would not possess the required properties, and therefore, could not permit hemoglobin formation and maturation of erythrocytes.

No conclusions can be drawn as to the possible interrelation of the increased copper content and other chemical changes of the blood observed. It has been reported that after hemorrhagic anemia glycolysis (21, 22) and respiration (23) of the blood cells are increased. Attempts have been made to correlate the increased respiration with the younger forms of erythrocytes appearing in the blood during recovery (24, 25), particularly with the reticulocytes (26). It has been pointed out by several investigators that glycolysis is activated by copper (27, 28). The observation that during recovery from hemorrhagic anemia the glutathione content of the blood is increased has been recorded frequently (29-33). It is interesting to note in this connection that, in contrast to the reduction of oxidized glutathione, the oxidation of the reduced compound is not enzymatic but metallic (34). The catalytic effect of iron and particularly of copper on the oxidation of cysteine is well known (35-37). A similar situation may apply to the accelerated oxidation of reduced glutathione by blood serum (38), although it has been shown that pure glutathione in vitro is not oxidized by free iron or copper (39). Whether the observation (40) that the oxidation-reduction potential of the bone marrow becomes more positive in hemorrhagic anemia is related to the chemical changes observed in the blood in this condition is a matter of conjecture.

Further intensive study on the chemical changes occurring in the blood during recovery from anemia may go far in explaining the necessity of copper for hemoglobin formation and the mechanism of its action.

SUMMARY

1. In pigs suffering from nutritional anemia due to iron and copper deficiency the copper content of the blood falls to extremely low levels.
2. Feeding 2 to 4 mg. of copper per day together with iron results in a very rapid and significant increase of the copper content of the blood.

3. When small amounts of copper are fed together with iron the increase in the copper content of the blood is only small and hematopoiesis is slow.

4. It is suggested that rapid, continued hematopoiesis cannot take place unless the copper content of the blood is maintained above a minimum level. This level may be about 20 micrograms per 100 cc. of blood of pigs.

5. The significance of these observations is discussed and it is suggested that the study of the chemical changes in the blood during recovery from severe nutritional anemia offers the most promising approach toward an understanding of the function of copper in blood formation.

BIBLIOGRAPHY

22. Meyerhof, O., Biochem. Z., 246, 249 (1932).
STUDIES ON THE COPPER CONTENT OF THE BLOOD IN NUTRITIONAL ANEMIA
M. O. Schultze, C. A. Elvehjem and E. B. Hart