Brief Report

Utilization of Iron by Scorbutive Guinea Pigs

By SACHCHIDANANDA BANERJEE AND ARDHENDU SEKHAR CHAKRABARTY

We reported earlier that scorbutive guinea pigs showed normocytic and normochromic anemia with normoblastic reaction of bone marrow, diminished plasma iron, decreased iron binding capacity of plasma, increased free erythrocyte protoporphyrin and a low sideroblast percentage with high hemosiderin content of bone marrow. These changes could not be attributed to inanition, intestinal hemorrhage, defective absorption of iron or intravascular hemolysis. The observations indicated defective utilization of iron in the scorbutive condition. Therapeutic response to iron in cases of anemia of scurvy, without simultaneous administration of ascorbic acid, had been reported which, however, could not be confirmed by others. The present report deals with the effect of administration of iron on the hematologic picture and tissue iron distribution in the scorbutive guinea pig.

Materials and Methods

Male guinea pigs, weighing between 250 and 300 Gm., were fed a scorbutogenic diet, 5 mg. ascorbic acid per animal per day and 2 drops of a concentrate of vitamins A and D twice a week. Animals which grew well were selected for experiment and divided into groups. (1) Scorbutive group: Animals were allowed to eat the scorbutogenic diet ad libitum and daily consumption of food of each animal was noted. Ascorbic acid was withdrawn. (2) Pair-fed normal group: Amount of food consumed by each animal of the scorbutive group was given on the next day to corresponding animal of this group. The animals continued to receive ascorbic acid. (3) Iron-treated scorbutive group: Animals were fed the scorbutogenic diet and in addition received orally 5 mg. ferrous sulfate per animal per day. Ascorbic acid was withdrawn. (4) Iron-treated normal group: Animals received scorbutogenic diet, ascorbic acid and ferrous sulfate until the animals of the scorbutive group developed scurvy.

Animals intended for iron treatment received the iron until they were sacrificed. Scurvy developed after the withdrawal of ascorbic acid for 22–24 days. Animals lost weight, were unable to move due to swelling of joints of the extremities, had anorexia and bleeding gums.

When guinea pigs of group (1) and (3) developed scurvy, blood samples were collected by cardiac puncture from animals of all groups in heparinised tubes for hematologic studies and estimation of plasma iron. The animals were killed by stunning, neck veins were cut for exsanguination and this was followed by decapitation. Tissues for iron studies were removed, blotted to remove adherent blood, washed three times with glass-distilled water, blotted again and a known amount of tissue taken for different estimations. The whole of the intestine was cut open to see the presence of hemorrhage.

Red blood corpuscles were counted by hemocytometry and packed cell volume was determined in Wintrobe tubes. Hemoglobin and plasma iron were estimated. Liver, spleen and intestine were fixed in neutral formal and paraffin sections prepared were stained for hemosiderin. Ferritin as "soluble iron fraction" and hemosiderin as "insoluble iron fraction" were estimated in the liver. Iron of liver, spleen, kidney and heart were estimated chemically. Hemosiderin of spleen, small intestine, bone marrow and liver was determined microscopically.

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RESULTS

None of the scorbutic animals treated with iron or without iron showed the presence of hemorrhage in the intestine.

Supplementation of iron to guinea pigs during the progress of scurvy did not improve or alter the type of anemia, but aggravated the condition. Hemoglobin level was lowered to 1.6 per cent, packed cell volume to 5 per cent and red cell count to 0.7 million per mm.$^3$ in some of the scorbutic animals receiving extra iron. Hypochromic and normocytic cells were still observed in the blood film.

Soluble iron fraction of liver which diminished in scorbutic guinea pigs did not change after treatment with extra iron. Insoluble iron fraction of liver which increased considerably in the scorbutic condition was further enhanced when scorbutic animals were treated with iron. Increased deposition of hemosiderin was found in spleen (fig. 1), small intestine (fig. 2) and bone marrow of scorbutic animals treated with iron. Iron treatment increased the total iron of tissues of scorbutic guinea pigs without increasing their plasma iron.

The detailed results are given in tables 1 and 2.

DISCUSSION

We have previously reported$^1$ that in scurvy normocytic anemia was the resultant effect of the presence of macrocytes and microcytes. If microcytes were due to associated iron deficiency, they should have disappeared after iron treatment of scorbutic animals. But microcytic hypochromic cells were still present in the peripheral blood and anemia continued to be normocytic and normochromic type even after treatment of the scorbutic guinea pigs with
Fig. 2.—Photomicrograph of section of small intestine from a scorbutic guinea pig treated with iron, showing deposition of hemosiderin in the villi. X 240.

iron. These observations indicated failure of iron therapy in the correction of scorbutic anemia.

Treatment with iron not only did not improve anemia of scurvy but made it more severe as indicated by the further diminution of hemoglobin, red blood cell count and the packed cell volume. The aggravation of anemia was not due to hemorrhage in the small intestine of the scorbutic animals treated with iron. All the animals received iron up to the time they were killed. None of the animals showed on examination the presence of hemorrhage in the intestine. The stool of these animals also did not show the presence of blood. Goldberg\textsuperscript{11} also did not observe any evidence of blood loss from the gut of his 55 patients suffering from scurvy. It therefore seems that factors other than intestinal hemorrhage were responsible for the aggravation of anemia in the scorbutic animals treated with iron. It has been reported\textsuperscript{12} that administration of iron resulted in a decrease of plasma ascorbic acid concentration. Bothwell et al.\textsuperscript{13} reported that Bantu subjects with scurvy had excessive iron deposits in tissues which modified the metabolism of ascorbic acid. It might be possible that the tissue depletion of ascorbic acid was more severe when the scorbutic animals received the iron treatment and as a consequence the anemia was aggravated. However this needs further investigation.

We reported\textsuperscript{1} that inanition was not responsible for the hypoferremia of scurvy. The present work demonstrated that hypoferremia of scurvy also could not be corrected by the oral administration of iron. This might be either due to defective absorption of iron or iron which was absorbed could not be retained in the plasma. Bothwell et al.\textsuperscript{13} reported that iron absorption was greater than normal in ascorbic acid deficiency. The deposition of hemosiderin in the villi of intestine and the increased tissue content of iron of the
Table 1.—Hematologic Picture of Guinea Pigs

<table>
<thead>
<tr>
<th></th>
<th>Scorbatic Guinea Pig (5)</th>
<th>Iron-Treated Scorbatic Guinea Pig (7)</th>
<th>Iron-Treated Normal Guinea Pig (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, Gm./100 ml</td>
<td>9.3 ± 0.63</td>
<td>5.9 ± 0.93</td>
<td>12.7 ± 0.55</td>
</tr>
<tr>
<td>Red blood cell, 10⁶/mm³</td>
<td>3.7 ± 0.18</td>
<td>2.5 ± 0.40</td>
<td>5.0 ± 0.24</td>
</tr>
<tr>
<td>P.C.V., %</td>
<td>31.8 ± 1.83</td>
<td>20.0 ± 2.93</td>
<td>40.7 ± 1.38</td>
</tr>
<tr>
<td>M.C.V., μ³</td>
<td>85.7 ± 1.58</td>
<td>79.7 ± 1.73</td>
<td>80.9 ± 2.02</td>
</tr>
<tr>
<td>M.C.H., γγ</td>
<td>24.8 ± 0.61</td>
<td>23.7 ± 1.09</td>
<td>25.0 ± 0.74</td>
</tr>
<tr>
<td>M.C.H.C., %</td>
<td>28.7 ± 0.85</td>
<td>30.2 ± 1.01</td>
<td>31.3 ± 0.82</td>
</tr>
</tbody>
</table>

Values = Mean ± S.E. Figures in parenthesis indicate number of animals.

Table 2.—Tissue Iron of Guinea Pigs

<table>
<thead>
<tr>
<th></th>
<th>Scorbatic Guinea Pig (5)</th>
<th>Iron-Treated Scorbatic Guinea Pig (7)</th>
<th>Pair-fed Normal Guinea Pig (6)</th>
<th>Iron-Treated Normal Guinea Pig (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble iron fraction of liver, μg./Gm.</td>
<td>25 ± 5.4</td>
<td>30 ± 6.5</td>
<td>60 ± 1.8</td>
<td>129 ± 15.1</td>
</tr>
<tr>
<td>Insoluble iron fraction of liver, μg./Gm.</td>
<td>62 ± 9.2</td>
<td>93 ± 13.6</td>
<td>38 ± 4.4</td>
<td>58 ± 6.7</td>
</tr>
<tr>
<td>Total iron, μg./Gm.:</td>
<td>Liver 104 ± 13.0</td>
<td>131 ± 13.6</td>
<td>110 ± 7.0</td>
<td>181 ± 9.2</td>
</tr>
<tr>
<td>Spleen 285 ± 39.7</td>
<td>1225 ± 131.7</td>
<td>196 ± 8.9</td>
<td>484 ± 42.0</td>
<td></td>
</tr>
<tr>
<td>Heart 45 ± 6.0</td>
<td>86 ± 9.3</td>
<td>68 ± 9.3</td>
<td>85 ± 8.5</td>
<td></td>
</tr>
<tr>
<td>Kidney 34 ± 6.0</td>
<td>58 ± 6.6</td>
<td>54 ± 6.4</td>
<td>60 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>Plasma iron, μg./100 ml.</td>
<td>78 ± 7.2</td>
<td>92 ± 9.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemosiderin:</td>
<td>Spleen ++++</td>
<td>++++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Liver +++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Intestine ++</td>
<td>+++</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow +++</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
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The average plasma iron of normal guinea pigs not treated with iron was 139 μg./100 ml.

scorbatic guinea pigs treated with iron also indicated that iron was absorbed from the gut. Anemia and hypoferremia therefore does not seem to be due to defective absorption of iron. Scorbatic guinea pigs were in iron balance and the increase in tissue hemosiderin of the animals was not due to intra-vascular hemolysis. The increased deposition of hemosiderin in tissues and bone-marrow of scorbutic guinea pigs treated with iron was, therefore, due to deposition of absorbed iron which could not be utilized. Normally iron was incorporated into ferritin, the soluble iron fraction of the liver. In scurvy this fraction diminished with concomitant increase in hemosiderin. Treatment of the scorbutic animals with iron did not increase ferritin of liver but enhanced the deposition of hemosiderin and increased the total iron content of tissues. This observation is in agreement with the suggestion of Mazur et al. about the specific role of ascorbic acid in the formation of ferritin. The role of as-
corbic acid in the enzymatic incorporation of iron into protoporphyrin for heme synthesis has also been suggested. Our observations are also in agreement with this suggestion. The presence of normocytic and normochromic anemia, the commonest form of anemia in scurvy, does not preclude the possibility of a deficiency in iron incorporation since microcytosis might be masked by macrocytosis.

SUMMARY

Administration of iron to guinea pigs during the progress of development of scurvy did not check the onset of anemia but enhanced it. Iron treatment also could not correct hypoferemia observed in the scurbutic condition although absorption of iron from the intestinal tract was not defective. The absorbed iron was deposited in tissues as hemosiderin. Ascorbic acid seems to be necessary for utilization of iron in the synthesis of hemoglobin.

SUMMARIO IN INTERLINGUA

Le administration de ferro a porcos de India in le curso del disveloppamento de scorbuto non arrestava le supervenientia de anemia sed promoveva lo. Therapia a ferro similemente non poteva corriger le hypoferremia observate in le condition scorbucit in despecto del facto que le absorption de ferro ab le vias intestinal non esseva defective. Le absorbite ferro esseva deponite in le tissus in le forma de hemosiderina. Il pare que acido ascorbic es necessari pro le utilisation de ferro in le synthese de hemoglobina.

REFERENCES

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