Reutilization of Iron in Anemia Complicating Malignant Neoplasms

By Farid I. Haurani, Kent Young and Leandro M. Tocantins

Anemias complicating malignant neoplasms may be secondary to blood loss, increased blood destruction, invasion of the hemopoietic organs or represent a side effect of the myelo-depressive agents used in the treatment of the neoplasm. Often none of these etiologic factors can be demonstrated. The anemia is usually mild to moderate in severity (8 to 10 Gm. per cent) and, less frequently, severe (7 Gm. per cent or less). The reticulocyte count is usually within normal limits. The red blood cells are normocytic. Mild to moderate degree of hypochromia of the red blood cells, not uniformly present, is often found upon examination of the peripheral blood smear. The bone marrow examination reveals normal cellularity with adequate erythroid activity. Erythrokinetic studies including radioiron clearance, red cell radioiron utilization, intestinal absorpton of iron and Cr51-tagged red blood cells survival often do not help in clarifying the mechanism of the anemia which remains obscure. Since the serum iron level is usually low despite increased iron stores and the anemia is not corrected by administration of iron orally, it was thought likely that a study of the degree and mode of reutilization of the iron already available in the body might be helpful in understanding the mechanism of the anemia. Freireich et al. has found poor reutilization of iron in dogs subjected to turpentine-induced inflammation.

This paper reports the results of the attempts at such a study in patients with malignant diseases, and the implications of the findings.

Methods

Differential cell counts on bone marrow were performed on erythrocytic elements encountered while counting 200 cells of the granulocytic series and the final count was expressed per 1000 such cells (excluding lymphocytes, monocytes, plasma cells and reticulum cells). Serum iron was measured by a modification of the method of Peterson, using 4,7-diphenyl-1, 10-phenanthroline. Blood volume was determined by the Cr51 tagged red cell method. Survival of Cr51-labeled red cells was determined by the method of Read et al. Mean red cell life (MCL) was calculated from the T/2 Cr51 value using the conversion table of Engstedt. Plasma iron turnover and red cell utilization of iron were measured according to the method of Huff. The radioiron as ferrous citrate was diluted with saline and injected intravenously. The plasma iron turnover production index was calculated from the formula given by Giblett et al. Details of the methods used have been published previously.

The radioiron-labeled hemoglobin solution was prepared as follows: 90 ml. of blood
Table 1.—The Characteristics of the Hemoglobin Solutions Injected into Patients and Normal Controls: The Radioactivity Was Determined at the End of the Study

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Hemoglobin (g%)</th>
<th>Volume (ml)</th>
<th>Hemoglobin Iron (mg./Rg.)</th>
<th>Radioactivity of Hemoglobin Iron (mg./Rg./hr.)</th>
<th>Fe59 Activity of Hemoglobin Solution (mg./Rg./hr.)</th>
<th>Per Cent of Fe59 Not Incorporated by Reticulocytes</th>
<th>Per Cent of Fe59 in the First 24-Hour Urine Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient #1</td>
<td>6.1</td>
<td>255</td>
<td>0.51</td>
<td>0.12</td>
<td>1583750</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td>Patient #2</td>
<td>2.8</td>
<td>50</td>
<td>0.32</td>
<td>0.11</td>
<td>1872958</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td>Patient #3</td>
<td>5.1</td>
<td>185</td>
<td>0.34</td>
<td>0.11</td>
<td>2367530</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>Patient #4</td>
<td>7.9</td>
<td>220</td>
<td>0.40</td>
<td>0.11</td>
<td>1308435</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Patient #5</td>
<td>2.3</td>
<td>100</td>
<td>0.15</td>
<td>0.09</td>
<td>7403175</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>Patient #6</td>
<td>7.8</td>
<td>166.5</td>
<td>0.37</td>
<td>0.13</td>
<td>1559606</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Patient #7</td>
<td>6.8</td>
<td>120</td>
<td>0.35</td>
<td>0.18</td>
<td>3734820</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Patient #8</td>
<td>3.7</td>
<td>112</td>
<td>0.21</td>
<td>0.11</td>
<td>8000048</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>Patient #9</td>
<td>4.2</td>
<td>138.5</td>
<td>0.25</td>
<td>0.11</td>
<td>1317412</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td>Patient #10</td>
<td>0.83</td>
<td>56</td>
<td>0.06</td>
<td>0.06</td>
<td>2665143</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>Control #1</td>
<td>2.5</td>
<td>65</td>
<td>0.13</td>
<td>0.12</td>
<td>12801670</td>
<td>3.7</td>
<td>0</td>
</tr>
<tr>
<td>Control #2</td>
<td>2.3</td>
<td>62</td>
<td>0.10</td>
<td>0.10</td>
<td>12801670</td>
<td>3.7</td>
<td>0</td>
</tr>
<tr>
<td>Control #3</td>
<td>2.8</td>
<td>200</td>
<td>0.13</td>
<td>0.03</td>
<td>3920024</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>Control #4</td>
<td>1.8</td>
<td>55</td>
<td>0.10</td>
<td>0.11</td>
<td>3071000</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>Control #5</td>
<td>4.7</td>
<td>200</td>
<td>0.23</td>
<td>0.23</td>
<td>1205200</td>
<td>8.0</td>
<td>0</td>
</tr>
</tbody>
</table>

was obtained from a patient with megaloblastic anemia receiving specific therapy at the peak of reticulocyte response (usually a patient with pernicious anemia receiving vitamin B12) or from a patient with hemolytic anemia and high reticulocyte count (more than 15 per cent). The blood was collected in a 100 ml. sterile silicone-coated bottle containing 10 ml. Acid Citrate Dextrose solution and 200 μc. of Fe59 (ferrous citrate), mixed well and transferred in 20 ml. aliquots to five silicone-coated sterile flasks where it was allowed to incubate for 4 to 6 hours at 37 C. in a Precision-Dubnoff water bath incubator shaker. The blood then was centrifuged and the plasma discarded. The red blood cells were washed three times or more with sterile saline to eliminate the radioiron that was not incorporated by the reticulocytes. The radioactivity of the last washing saline was never more than 8 per cent of the total radioactivity, the average being 2.2 per cent. The packed red blood cells were resuspended in sterile saline to give a hematocrit of about 40 per cent. Finally the red cells were hemolyzed by adding 1 part of red cell suspension to 2.5 of sterile distilled water. The final hemoglobin solution was tested for sterility before injection into the recipient.

Recipients for the hemoglobin solution were selected if they fulfilled the following conditions: (a) a steady state of the type of anemia under study, and (b) blood group compatibility with the hemoglobin solution available at the time. Once these conditions were met, the hemoglobin solution was slowly (15 drops/min.) injected intravenously over a period of a few hours (table 1). In terms of hemoglobin iron, the rate of injection was never above 0.18 mg. per Kg. per hour except that in one patient it was 0.46. The average hemoglobin iron load was 0.24 mg. per Kg. (table 1). The total Fe59 activity of the hemoglobin solution at the time the study was completed varied from 1,205,200 to 12,901,670 counts per 3 minutes with an average of 4,519,641 counts per 3 minutes. Urine was collected during the first 24-hour post-injection period. No Fe59 activity was detected in any of the specimens tested. Blood specimens were collected from the recipient at weekly intervals for 3 weeks. At the end of this period, plasma radioiron clearance and radioiron red cell utilization were performed as well as determination of Cr51-tagged red cell survival.
and total blood volume. In the determination of the utilization of iron, correction for the Fe\textsuperscript{59} activity from the reutilization study was made, assuming that during the 3rd week maximal reutilization is reached. The total radioactivity used in the utilization study was in most cases four to five times that of the reutilization study, and thus the latter could not account for more than 20 per cent of the utilization percentage, most of which had been already corrected.

### Results

Table 2 depicts the hematologic data in 10 patients studied. Five patients had Hodgkin's disease and the others had lymphosarcoma, fibrosarcoma with metastasis, carcinoma of the small intestine with metastasis, uterine carcinoma with metastasis and multiple myeloma. Their blood hemoglobin levels varied between 5.7 Gm. per cent and 10.0 Gm. per cent. The mean corpuscular hemoglobin concentration ranged between 25.4 and 30.2 per cent. The absolute reticulocyte count was normal or slightly elevated (not more than twice normal). The bone marrow was of normal cellularity, and normal myeloid:erythrocyte ratios were present in all patients except one (Case #3) who had myeloid hyperplasia. The serum iron level was low in four patients, low normal in five and normal in one. The serum total iron-binding capacity was low in seven patients, low normal in two and normal in one. The Fe\textsuperscript{59} T/2 clearance rate was faster than normal in nine patients and in one patient it was normal. However, the plasma iron turnover index was within normal limits except for Cases 2, 9 and 10 in whom it was low and in Case 5 (slightly elevated). Maximum utilization of iron was normal in all patients. Survival of the patients' red blood cells by the Cr\textsuperscript{51}-tagging technic was slightly diminished in almost all patients. In one patient (Case #7) it was moderately shortened, the destruction rate being 3.6 times normal.

Three of the controls were patients with pernicious anemia in remission. The other two were patients without anemia or any other hematologic problem. The controls were tested only for reutilization of iron.

Figure 1 shows the maximum reutilization of iron in this group of patients. In all patients the curves for reutilization of iron are lower than those for the normal controls. The difference between the two groups becomes evident after the 7th day and more marked on the 21st day. Maximal reutilization of iron in the normal group is 70 per cent or more at about the 3rd week whereas at that time in patients with malignant disease it varies between 10 per cent and 50 per cent depending on the severity of the anemia. Figure 2 shows the good correlation observed when the hemoglobin concentration (Gm. per cent) of each patient is plotted against his percentage of reutilization of iron.

### Discussion

The mammalian reticulocytes have been shown to incorporate radioactive iron in vitro for hemoglobin synthesis.\textsuperscript{14-18} Iron, aminoacids, glucose and transferrin are essential for this in vitro synthesis of hemoglobin.\textsuperscript{14,18} The incorporation of Fe\textsuperscript{59} into hemoglobin at a given temperature depends also on the duration of the exposure and the number of reticulocytes.\textsuperscript{15,16} In systems containing only labeled iron, iron binding protein and washed reticulocytes suspended in
Table 2.—Hematologic Data on Patients with Anemia Complicating Malignant Disease

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Reticulocyte Count (%)</th>
<th>Red Blood Cells (10^6 per cu. mm.)</th>
<th>Hemoglobin Conc. (Gm. %)</th>
<th>Mean Corpuscular Hemoglobin Conc. (%)</th>
<th>Serum Iron (µg. % L.)</th>
<th>Total Iron Binding Capacity (µg. %)</th>
<th>Fe^3+ T/2 Clearance (mina.)</th>
<th>Plasma Iron Turnover Index</th>
<th>Maximum Utilization of Fe^3+ (%)</th>
<th>Maximum Reutilization of Fe^3+ (%)</th>
<th>Mean Cell Life-Cycle (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hodgkin's disease</td>
<td>3.2</td>
<td>2.9</td>
<td>8.4</td>
<td>27.1</td>
<td>35</td>
<td>185</td>
<td>25</td>
<td>1.04</td>
<td>90</td>
<td>23</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>Hodgkin's disease</td>
<td>3.0</td>
<td>2.3</td>
<td>6.2</td>
<td>25.8</td>
<td>23</td>
<td>173</td>
<td>19</td>
<td>1.00</td>
<td>87</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Hodgkin's disease</td>
<td>2.8</td>
<td>3.6</td>
<td>8.8</td>
<td>26.6</td>
<td>34</td>
<td>385</td>
<td>64</td>
<td>0.40</td>
<td>85</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>Hodgkin's disease</td>
<td>3.0</td>
<td>2.9</td>
<td>8.4</td>
<td>29.0</td>
<td>65</td>
<td>41</td>
<td>1.20</td>
<td>77</td>
<td>42</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>Hodgkin's disease</td>
<td>3.2</td>
<td>3.2</td>
<td>9.0</td>
<td>27.9</td>
<td>64</td>
<td>164</td>
<td>23</td>
<td>1.9</td>
<td>80</td>
<td>40</td>
<td>120</td>
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<tr>
<td>6</td>
<td>Lymphosarcoma</td>
<td>1.6</td>
<td>4.0</td>
<td>10.0</td>
<td>27.0</td>
<td>81</td>
<td>246</td>
<td>25</td>
<td>2.30</td>
<td>90</td>
<td>43</td>
<td>88</td>
</tr>
<tr>
<td>7</td>
<td>Fibrosarcoma with metastasis</td>
<td>1.4</td>
<td>3.0</td>
<td>7.1</td>
<td>29.6</td>
<td>47</td>
<td>127</td>
<td>65</td>
<td>1.30</td>
<td>68</td>
<td>39</td>
<td>33</td>
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<tr>
<td>8</td>
<td>Carcinoma of small intestine with metastasis</td>
<td>1.2</td>
<td>3.6</td>
<td>8.1</td>
<td>25.4</td>
<td>50</td>
<td>265</td>
<td>29</td>
<td>1.30</td>
<td>68</td>
<td>39</td>
<td>33</td>
</tr>
<tr>
<td>9</td>
<td>Uterine carcinoma with metastasis</td>
<td>3.1</td>
<td>2.5</td>
<td>5.7</td>
<td>27.0</td>
<td>34</td>
<td>159</td>
<td>59</td>
<td>0.40</td>
<td>70</td>
<td>10.5</td>
<td>58</td>
</tr>
<tr>
<td>10</td>
<td>Multiple myeloma</td>
<td>1.2</td>
<td>4.1</td>
<td>10.0</td>
<td>30.2</td>
<td>55</td>
<td>255</td>
<td>99</td>
<td>0.43</td>
<td>68</td>
<td>50.3</td>
<td>107</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>1.0</td>
<td>4.5–5.0</td>
<td>14.5</td>
<td>32–34</td>
<td>50–150</td>
<td>270–400</td>
<td>60–120</td>
<td>1.0</td>
<td>&gt; 75</td>
<td>70</td>
<td>120</td>
</tr>
</tbody>
</table>
saline and incubated at 37 C. for 2 hours, Jandl and his co-workers observed that from 30 to 80 per cent of the reticulocyte Fe was incorporated into heme. Erslev, using a system in which conditions were similar to ours, found 70 per cent of the bone marrow Fe incorporated into hemoglobin. In experiments using Borsook's modification of Roche's method for isolation of hemoglobin, and by electrophoresis and strip scanning of the radioactive hemoglobin solution, we found 80 to 90 per cent of the reticulocyte Fe to be incorporated into hemoglobin.

The bone marrow of an average normal adult needs about 25 mg. of iron daily. Of this only 1.5 mg. of iron is supplied by the food; therefore, more than 90 per cent of the bone marrow daily iron needs must come through reutilization of iron from senescent red blood cells. The observation that utilization of radioiron in normal individuals is more than 70 per cent indicates that the plasma iron compartment, which is normally about 2 to 3 mg. at a given moment, is not in direct communication with storage iron. The intravenous injection of radioiron, already tagged to hemoglobin, into normal
individuals measures the reutilization of iron, namely the iron released from senescent red blood cells capable of re-entering the plasma iron compartment from the reticuloendothelial system. The fact that, in normal individuals, the reutilization of iron as determined by this study (fig. 1) is 70 per cent or more does also indicate that the active storage pool of iron at a given moment is not greatly different in amount than the plasma iron compartment. The delay in obtaining the maximal reutilization of iron (21 days) as compared to the maximal utilization of iron (10 days) may reflect the additional period required for release of iron from hemoglobin and delivery to the circulation by the reticuloendothelial cells. Noyes et al., using a different method, found that about 55 to 60 per cent of injected red cell iron is reutilized by the normal individual around the 3rd week. The slight difference between our values and theirs might be due to the fact that in this study a small fraction of the reticulocyte iron is not incorporated into heme. The utilization of Fe59 and reutilization of hemoglobin Fe59 are not comparable, at least because the iron loads injected for the determination of each are different in amounts. However, each should be compared to its normal range. The retention of erythrocyte iron by the reticuloendothelial system in normal individuals remains stable if variations of the dosage of the erythrocyte iron loads are kept between 0.15 and 0.95 mg. per Kg. of body weight.

In this group of patients the radioiron, when injected intravenously as ferrous citrate, cleared rapidly from the circulation; however, since the serum iron levels were low, the plasma iron turnovers came within normal limits in most of the patients. The utilization of radioiron by the bone marrow in these patients also appeared normal. These findings, coupled with the appearance of the peripheral blood and bone marrow, bears some similarity to simple iron deficiency anemia except in two aspects. First, the serum iron binding capacity...
is low or low normal in the anemias under discussion, whereas in the ordinary iron deficiency anemia, be it the result of poor iron intake, poor iron absorption or chronic blood loss, the serum iron binding capacity is elevated. Second, the anemia associated with malignant diseases seldom responds to iron in the amounts used in the treatment of simple iron deficiency. Total erythropoiesis as determined by plasma iron turnover index and bone marrow aspiration is almost normal, if not normal, in simple iron deficiency, but if the level of hemoglobin is considered, these values may be construed to represent relative failure of the bone marrow. The same argument could be applied also to the anemia complicating malignancy.

The study of iron reutilization in this group revealed another difference between simple iron deficiency anemia and the anemia of malignancy. The defective reutilization of iron in these patients probably accounts primarily for their anemia. As mentioned earlier, the reutilization of iron provides a greater supply of iron to the bone marrow per unit of time than iron absorbed from the gastrointestinal tract or parenteral sources.

The mechanism responsible for this defective reutilization of iron is not clear. The serum iron-binding protein must be involved since the level of this protein is often low normal, if not low in these patients. Mazur and his associates have studied in vitro and in vivo the release and storage of iron by tissue ferritin. The xanthine dehydrogenase system seems to enhance the release of iron from tissue cells. Purines capable of being oxidized by this enzyme to uric acid could in turn reduce iron in the ferritin-ferric complex and raise the plasma iron level. Factors that favor storage of ferritin in the tissue cells seem to be related to the increased oxidative metabolism of the tissues. The defect in reutilization of iron in some of the anemias of malignancy could be related to any of the factors which decrease the release of iron from tissues or increase tissue iron storage. This obviously requires further study and elucidation.

Anemia resulting from defective reutilization of iron is not peculiar to malignant disease. Preliminary studies indicate that a similar defect in reutilization of iron is observed in the anemias associated with infection. A similar situation may also exist in certain chronic inflammatory states such as sarcoidosis and rheumatoid arthritis.

**Summary**

A study of reutilization of iron by means of Fe$^{59}$-tagged hemoglobin solution given to normal individuals yielded a rate of reutilization of 70 per cent or more, evidence perhaps that the active storage pool of iron at a given moment is not larger than the plasma iron compartment. Reutilization of iron in patients with anemia complicating malignancy disease has shown a significant decrease which correlates well with the severity of the anemia.

**Summario in Interlingua**

Un studio del re-utilisation de ferro, executate con le uso de un solution de hemoglobina marcate con Fe$^{59}$ le qual esseva administrate a subjectos normal,
monstrava un re-utilisation de 70 pro cento o plus. Isto significa possibilmente que le active reservoir de ferro a un momento particular non es plus grande que le compartimento de ferro in le plasma. Le re-utilisation de ferro in patientes con anemia occurrente como complication de morbo maligne ha monstrate un significative declino, lo que es ben correlationate con le severitate del anemia.

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REFERENCES

REUTILIZATION OF IRON


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