Radioactive Iron Studies in Patients with Iron Deficiency Anemia with Concurrent Abnormal Hemolysis

By M. C. Verloop, M. Van der Wolk and A. J. Heier

Occasionally patients with hypochromic anemia and signs of iron deficiency are encountered who show suboptimal response to iron administration. A possible cause of this is blood loss, including the loss of blood into organs, as in pulmonary hemosiderosis. In the absence of blood loss, the cause may be a disturbance in iron absorption from the gastrointestinal tract. Infections, too, can have an unfavorable effect on iron incorporation in hemoglobinopoiesis; the same holds true for vitamin C deficiency (Parsons and Cary Smallwood). In the absence of such changes, it is more difficult to discover the cause of poor response to iron therapy. Zetterström and Delava described two children with hypochromic anemia and a low serum iron concentration, refractory to iron therapy. There was no steatorrhea, but the absorption of radioactive iron was insufficient. Intravenously given radioactive iron, moreover, was poorly utilized in hemoglobinopoiesis. No signs of abnormal hemolysis were found.

We have had occasion to examine two adult women with severe anemia who showed, besides signs of iron deficiency, a markedly reduced life-span of their red cells. Transfused normal red cells, moreover, disappeared from the circulation rapidly. In these patients, extracorpuscular factors in organs or plasma seemed to cause abnormal hemolysis.

Methods

Iron metabolism.—The serum iron concentration and the iron in urine were determined by the method of Heilmeyer and Plötzner, as modified subsequently by Lederer and de Maeschalck. The serum iron-binding capacity was determined according to the method of Rath and Finch. Iron in bone marrow smears was stained by a modification of the method of Kaplan, Zuelzer and Mouriand. Plasma iron disappearance and incorporation of Fe in the circulating hemoglobin were determined following intravenous injection of 10 µc. of Fe as iron citrate (Wetherley-Mein, Hutt, Langmead and Hill; Bothwell, Callender, Mallett and Witts). The plasma volume was measured on the basis of the calculated erythrocyte volume, determined after injection of erythrocytes labeled with Cr, and the corrected hematocrit value (Chaplin and Mollison). The plasma iron turnover was expressed in mg./100 ml. per 24 hours (whole blood). The red cell iron turnover was determined according to Huff et al.

Red cell life span.—The osmotic resistance of the red cells—fresh and after 24 hours’ incubation at 37 °C.—was measured quantitatively. The quantitative fecal excretion of urobilinogen was determined according to Heilmeyer and Krebs. If necessary, the hemolytic index was calculated. Red cell life span was estimated after labeling with radioactive chromium. We found the mean normal half-value time of Cr to be 28.3 days ± 2.3 days. After autotransfusion of red cells labeled with Cr, surface radioactivity

From the Hematological Laboratory, Geneeskundige Universiteitskliniek, Utrecht (Netherlands).

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was measured over the heart, liver and spleen; the indexes of Jandl were calculated.\textsuperscript{19} The plasma Hb concentration was calculated according to Flink and Watson.\textsuperscript{12}

Supplemental investigations.—Additional measurements included alkali-resistant hemoglobin,\textsuperscript{8} paper electrophoretic\textsuperscript{33} and starch electrophoretic analysis of hemoglobin.\textsuperscript{24} The red cell protoporphyrin content,\textsuperscript{31,33} the serum vitamin C level,\textsuperscript{30} and the serum copper concentration\textsuperscript{9} were also determined in some instances.

CASE HISTORIES AND RESULTS

Patient 1

Miss M., born December 16, 1924, had a negative history up to 1944, after which she complained of fatigability, with repeatedly observed anemia. In 1954 the Hb concentration was 10 Gm./100 ml., and the red cell count 4,000,000; subsequently the anemia showed gradual aggravation. In August 1956 the Hb concentration was 5.2 Gm./100 ml. The diet had always been adequate. There were no signs of a hemorrhagic diathesis, and no manifest blood loss occurred. The menstrual cycle was five weeks, but menstruation was sometimes rather abundant. There was no familial history of anemia; examination of relatives was impossible.

Physical examination of this thin patient (height 1.65 M., weight 44 Kg.) revealed nothing abnormal, particularly no enlargement of lymph glands, hepatomegaly or splenomegaly. The temperature was normal.

Hematologic examination between August 1956 and August 1959 showed an Hb concentration fluctuating between 4 and 7 Gm./100 ml. without treatment. The anemia was sometimes hypochromic, sometimes normochromic. The remaining data on the blood picture are given in table 1 and figure 1.

The urine was normal. The feces contained no occult blood; there were no digestive disorders, particularly no steatorrhea. Fractional gastric examination yielded normal acid values. Blood chemistry results included a normal bilirubin concentration; other tests of liver function were normal. Further data are presented in table 2. Endocrinologic examination revealed a low basal metabolism (—15 per cent). Thyroid function tests with radioactive iodine showed a low normal uptake (maximum 33.4 per cent) into the thyroid. The blood cholesterol level was 181 mg./100 ml. The fasting blood sugar level was 93 mg./100 ml., and a blood sugar tolerance curve showed a normal course. X-ray examination of the heart, lungs, kidneys and spleen was reported to be normal.

Special investigations of the anemia.—Cytologic examination of bone marrow specimens showed a hyperplastic red cell system and reversal of the ratio between erythropoiesis and leukopoiesis (3:2). There was an increase in basophilic erythroblasts; no other changes were found. The periodic acid-Schiff (PAS) staining reaction was negative.

Hemoglobin findings. The alkali-resistant fraction of the hemoglobin was 0.4 per cent. Paper electrophoresis and starch electrophoresis revealed no anomalous hemoglobins.

Iron metabolism. The serum iron concentration was invariably decreased, even after blood transfusions and during therapy with readily absorbed oral iron (table 3). The latent iron-binding capacity of the serum was either normal or increased. After administration of 176 mg. of iron as ferrous chloride by the oral route, the serum iron concentration showed an increase from 20 to 200 $\mu$g./100 ml. within 2 hours (table 2).

Iron staining of bone marrow specimens revealed iron pigment in the bone marrow fragments in a form such as can be seen following parenteral administration of iron (fig. 2). No stainable iron was otherwise demonstrable in the reticuloendothelial system; the sideroblast count was below normal.

The excretion of iron in the 24 hour urine was low, less than 0.5 mg.

Clinical course.—Oral iron therapy and administration of prednisone, copper sulfate and pyridoxine failed to produce a rise in Hb concentration (fig. 3). Subsequent therapy included oral administration of folic acid, cobalt chloride and methionine, and injections of vitamin B$_{12}$, B complex and C, and phenyl propionate, again with no effect on the anemia. Only parenteral iron administration produced an unmistakable if transient rise in Hb concentration (fig. 3).
Fig. 1 (at top).—Photomicrograph of blood film showing hypochromia, fully hemoglobinized cells and small poikilocytes, before therapy. ×750; reduced.

Fig. 2 (at bottom).—Photomicrograph of bone marrow film stained for iron. Numerous iron granules were present, scattered in and among the reticulum cells. These granules were of the same size and appearance as the iron "crystals" appearing in marrow shortly after parenteral iron therapy. No other stainable iron was present.
Table 1.—Hematologic Data

<table>
<thead>
<tr>
<th></th>
<th>Patient I</th>
<th>Patient II</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (Gm./100 ml.)</td>
<td>7</td>
<td>4.2</td>
<td>12.6–15.4</td>
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<tr>
<td>Red blood cells (cu.mm.)</td>
<td>3.1 x 10^6</td>
<td>2.3 x 10^6</td>
<td>(4–5) x 10^6</td>
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<tr>
<td>Color index</td>
<td>0.73</td>
<td>0.59</td>
<td>0.9–1.1</td>
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<tr>
<td>Hematocrit (%)</td>
<td>23</td>
<td>17</td>
<td>36–46</td>
</tr>
<tr>
<td>M.C.H. (µg.)</td>
<td>30</td>
<td>25</td>
<td>32–38</td>
</tr>
<tr>
<td>M.C.V. (cu.mm.)</td>
<td>74</td>
<td>78</td>
<td>78–98</td>
</tr>
<tr>
<td>M.C.D. (µ)</td>
<td>7.9</td>
<td>8.0</td>
<td>6.7–7.7</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>0.5–11.5</td>
<td>2–14</td>
<td>0.2–2</td>
</tr>
<tr>
<td>White blood cells (cu.mm.)</td>
<td>3800</td>
<td>4400</td>
<td>4.1–10,000</td>
</tr>
<tr>
<td>Platelets (cu.mm.)</td>
<td>284,000</td>
<td>151,000</td>
<td>150–350,000</td>
</tr>
<tr>
<td>Sed. rate (mm./hr.)</td>
<td>7</td>
<td>4</td>
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Table 2.—Some Data on Blood Chemistry

<table>
<thead>
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<th></th>
<th>Patient I</th>
<th>Patient II</th>
<th>Mean normal values</th>
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<tr>
<td>Fasting</td>
<td>20</td>
<td>38</td>
<td>120 ± 26</td>
</tr>
<tr>
<td>Serum iron</td>
<td>200</td>
<td>211</td>
<td>239 ± 69</td>
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<tr>
<td>2 hr. after 'Iron abs. test'</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Latent iron-binding capacity</td>
<td>420</td>
<td>310</td>
<td>222 ± 40</td>
</tr>
<tr>
<td>Serum copper (µg./100 ml.)</td>
<td>145</td>
<td>233</td>
<td>73–135</td>
</tr>
<tr>
<td>Vitamin C (mg./1000 ml.)</td>
<td>6</td>
<td>4</td>
<td>4–12</td>
</tr>
<tr>
<td>Free erythrocyte porphyrins (µg./100 ml. of packed cells)</td>
<td>56</td>
<td>48</td>
<td>26–56</td>
</tr>
</tbody>
</table>

Patient 2

Miss v. H., born December 24, 1923, received sanitarium treatment for pulmonary tuberculosis during the period 1949–1953. Anemia was occasionally observed during that period. No pulmonary changes have since occurred. The anemia, however, became exacerbated, and the Hb concentration fluctuated between 4.2 and 6 Gm./100 ml., no therapy being given. There was nothing to suggest any dietary deficiency. There were no signs of a hemorrhagic diathesis or of manifest blood loss. Menstruation occurred every 4 to 5 weeks; bleeding was not copious. No case of anemia in the family was known. Examination of relatives was unfortunately impossible.

Physical examination (height 1.53 M., weight 41 Kg.) revealed no anomalies, particularly no enlargement of lymph glands, liver or spleen. The temperature was normal.

Examination of the blood revealed marked hypochromic microcytic anemia (table 1).

The urine was normal. The feces contained no occult blood nor evidence of steatorrhea. Fractional gastric examination revealed normal acid values. Blood chemistry included a normal bilirubin concentration; liver function tests in the blood were likewise normal. Further findings are listed in table 2. Endocrinologic examination. The basal metabolism was —11 per cent. Radioactive iodine uptake by the thyroid was 31.4 per cent. The fasting blood sugar value was normal: 95 mg./100 ml. X-ray examination. X-ray films of the skull, heart, lungs, esophagus, stomach, duodenum, large and small intestine and spleen were interpreted as showing no abnormalities.

Special investigations of the anemia.—Cytologic examination. The bone marrow
Fig. 3 (at top).—Patient 1. Effect of therapy on hemoglobin level and reticulocyte count. Only intravenous administration of iron caused an unmistakable if very brief transient increase in hemoglobin concentration.

Fig. 4 (at bottom).—Patient 2. Effect of therapy on hemoglobin level and reticulocyte count. When the hemoglobin level was raised by 4 blood transfusions to 11.6 Gm. 100 ml., the reticulocyte count dropped nearly to zero. Afterwards the hemoglobin level rapidly decreased again, showing abnormal hemolysis of the transfused normal red cells. During oral administration of a large quantity of iron, there was an increase in hemoglobin concentration. Interruption of treatment caused a decrease. Intramuscular injections of iron caused a very rapid increase in hemoglobin concentration, followed by an even more rapid decrease.

Aspirate showed a hyperplastic red cell system (the ratio between erythropoiesis and leukopoiesis was 1:1) with a relative increase in basophilic erythroblasts. No other changes were seen. The PAS reaction was negative.

Hemoglobin findings. The alkali-resistant hemoglobin fraction was 0.8 per cent. No abnormal hemoglobins were demonstrable either by paper or by starch electrophoresis.

Iron metabolism. Serum iron values nearly always showed a considerable decrease (table 3). After ingestion of 300 to 350 mg. per day of a highly absorbable type of iron, fasting serum iron values were 40 and 47 μg./100 ml. The serum iron value returned to 38
Table 3.—Serum Iron Levels Before and After Therapy

<table>
<thead>
<tr>
<th></th>
<th>Fasting serum iron (μg./100 ml.)</th>
<th>Latent iron bind. cap. (μg./100 ml.)</th>
<th>Saturation (%)</th>
<th>Therapy</th>
</tr>
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<tbody>
<tr>
<td>Pt. 1.</td>
<td>20</td>
<td>420</td>
<td>5</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>400</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>325</td>
<td>15</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>235</td>
<td>21</td>
<td>After 3 blood transfusions</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>255</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>400</td>
<td>15</td>
<td>Iron by mouth</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>325</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>275</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>300</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Pt. 2.</td>
<td>21</td>
<td>310</td>
<td>6</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>225</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>205</td>
<td>27</td>
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<td></td>
<td>28</td>
<td>275</td>
<td>9</td>
<td>Vitamin C</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>220</td>
<td>15</td>
<td>Iron by mouth</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>235</td>
<td>17</td>
<td>1 week after 2 Gm. of iron I.M.</td>
</tr>
<tr>
<td></td>
<td>82</td>
<td>200</td>
<td>29</td>
<td>2 weeks after 2 Gm. of iron I.M.</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>210</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

μg./100 ml. two weeks after completion of a course of I.M. injections of 2 Gm. of iron. Ingestion of 176 mg. ferrous iron was followed two hours later by an increase in serum iron from 38 to 211 μg./100 ml. (table 2). The latent iron-binding capacity of the serum amounted to 200 to 310 μg./100 ml.

Iron staining of the bone marrow specimens revealed fragments of iron which seemed to originate from previously injected colloidal iron. No other stainable iron was demonstrable in the bone marrow fragments; the sideroblast count was low. The excretion of iron in the 24 hour urine was less than 0.5 mg.

Clinical course.—The anemia remained refractory to administration of pyridoxine, vitamin C and prednisone (fig. 4); previous injections of vitamin B₁₂ and oral administration of folic acid had been ineffective. The Hb concentration rose when the patient ingested a considerable quantity of iron; interruption of this therapy was followed by rapid exacerbation of the anemia (fig. 4). In April 1959 the patient received 2 Gm. of iron by intramuscular injections, after which the Hb concentration increased from 6 to 10.6 Gm./100 ml. After 2 weeks this value decreased again from 10.6 to 3.7 Gm./100 ml.

Epicrosis.—Both patients showed anemia with the signs of iron deficiency. The red cells were in part hypocromic and the average Hb concentration of the cells was low. The serum iron level was invariably low, with sometimes a normal but as a rule an increased latent iron-binding capacity of the serum. The serum copper level was slightly increased. Iron staining of the bone marrow was not uncommon in patients with iron deficiency. A quantity of iron pigment as found in these cases more than a year after iron injections, however, is unusual. No other stainable iron was demonstrable in the reticuloendothelial system, and the sideroblast count was below normal.

In the first patient the Hb concentration could be increased only by parenteral iron therapy (fig. 3). In the second patient the Hb rose after oral administration of a large quantity of ferrous iron, to a maximum of 9 Gm./100 ml. (fig. 4). The iron prescribed was readily absorbed in both cases (table 2). No blood loss was demonstrable, nor were there any signs of pulmonary hemosiderosis or hemoglobinuria. No infection existed.
Special Investigations

Investigation with the aid of radioactive iron (Fe\(^{59}\)).—Both patients showed a good balance between production and breakdown of red cells at a Hb concentration between 4 and 6 Gm./100 ml. In view of the severe anemia, it was unfortunately impossible to investigate the iron metabolism with the aid of radioactive iron throughout a prolonged period during a steady state. Each of the patients received two intravenous injections of Fe\(^{59}\), after which the red cell utilization percentage was determined. The shape of the curves obtained was influenced by treatment with iron.

While patient 1 was receiving oral iron therapy, she was also given 10 μc. of Fe\(^{59}\) intravenously. There was a high plasma clearance rate with a half-value time of 13 minutes. The radioactive iron was incorporated into the red cells more rapidly than normal (it was demonstrable in the red cells 100 minutes after the intravenous injection!); a maximum utilization percentage of 87 was reached after 9 days (fig. 5). The saturation percentage fell to 35 in the following weeks, after which no further determinations were made. Thus, abnormal hemolysis was brought to light by iron administration. The Fe\(^{59}\) liberated in this process appeared to be insufficiently used, if at all, for reutilization in the bone marrow. The therapeutic iron was preferred for hemoglobinopoiesis.

Almost a year later, 10 μc. of Fe\(^{59}\) was again intravenously injected (fig. 6).

![Fig. 5. — A, Plasma disappearance of Fe\(^{59}\) in patient 1; the half-disappearance time from the circulation was 13 minutes. Normal range (shown hatched) 60 to 140 minutes. The disappearance rate was not exactly exponential.

B, Utilization of Fe\(^{59}\) for hemoglobin synthesis. The maximum utilization was 87 per cent. Normal range (shown hatched) 78 to 90 per cent (Bothwell et al.\(^5\)). In the following weeks the utilization percentage decreased steadily, showing increased hemolysis. The patient received oral iron therapy.]
Fig. 6.—A, Plasma disappearance of Fe$^{59}$ in patient 1; the half-disappearance time from the circulation was 12 minutes. Normal range (shown hatched) 60 to 140 minutes. The disappearance rate was not exactly exponential.

B, Utilization of Fe$^{59}$ for hemoglobin synthesis. The maximum utilization was 95 per cent. Normal range (shown hatched) 78 to 90 per cent. Without therapy the utilization percentage remained constant for about two weeks. When medicinal iron was administered intravenously, the utilization percentage of Fe$^{59}$ decreased to 26. This curve revealed a shortened life span of the patient's red cells. During the steady state before iron therapy, there seemed to be reutilization of the radioactive iron liberated from destroyed erythrocytes. During iron therapy no reutilization or a decreased reutilization of radioactive iron in hemoglobinopoiesis seemed to occur.

It had been previously established that the Fe$^{59}$ injected in 1958 was no longer demonstrable in the red cells. Patient 1 had had no more iron therapy by the oral route during the 6 preceding months; her condition seemed to be balanced. Without treatment she maintained a Hb concentration of 4 to 5.8 Gm./100 ml. The plasma clearance rate was considerably accelerated (t $^{1/2}$ 12 minutes). The maximum utilization by the red cells had increased to 95 per cent. After a mild decrease to 83 per cent, it remained constant throughout the steady state. The Fe$^{59}$ plasma iron turnover was 4.8 mg./100 ml. whole blood per 24 hours (normal value less than 1 mg.). This calculation was not entirely accurate, however, because the disappearance of the radioactive iron from the plasma was not purely exponential. The red cell Fe$^{59}$ turnover during this steady state was 3.9 mg./100 ml. whole blood per 24 hours, that is, more than four times the normal value. Under intravenous iron therapy the utilization percentage fell to 26 within a few weeks (fig. 6). When the patient's condition was balanced without iron therapy, the Fe$^{59}$ liberated in
accelerated hemolysis was used in the production of new hemoglobin in the bone marrow. As soon as iron was administered, reutilization of the Fe$^{59}$ liberated was lacking or insufficient.

In the second patient, the maximum utilization by the red cells during oral iron therapy was 85 per cent of 10 $\mu$ c. of Fe$^{59}$ intravenously injected. As in the first patient, the utilization percentage decreased in the following weeks due to accelerated erythrolysis and insufficient reutilization of Fe$^{59}$ in hemoglobinopoiesis (fig. 7). Almost a year later, radioactive iron was no longer demonstrable in the blood. Another intravenous injection of radioactive iron was given, 6 days after completion of a course of iron injections (fig. 8 and fig. 4). There was an accelerated plasma clearance rate with a $t_{1/2}$ of 15 minutes. Incorporation into the red cells was more rapid than normal, but the maximum utilization percentage was below normal (66 per cent). This could be expected immediately after intramuscular administration of 2 Gm. of iron.$^{11}$

In the course of the following days the red cell Fe$^{59}$ percentage decreased to 33. Within 5 days, at least 50 per cent of the labeled red cells was lost again. This—since there was no blood loss—indicated marked erythrolysis. Taking into account the possibility that new red cells labeled with Fe$^{59}$ may have entered the circulation in these 5 days, we find that the red cell life span at the time cannot have been more than 10 days. In the following weeks the Fe$^{59}$ utilization percentage showed a further decrease to about 12 per cent (fig. 8).

![Graph](https://example.com/graph.png)

**Fig. 7.—** A, Plasma disappearance of Fe$^{59}$ in patient 2; the half-disappearance time from the patient's circulation was 10 minutes. Normal range (shown hatched) 60 to 140 minutes. The disappearance rate was not exactly exponential.

B, Utilization of Fe$^{59}$ for hemoglobin synthesis. The maximum utilization was 85 per cent. Normal range (shown hatched) 78 to 90 per cent. In the following weeks the utilization percentage decreased, showing increased hemolysis. The patient received oral iron therapy at that time.
Fig. 8.—A. Plasma disappearance of Fe\textsuperscript{59} in patient 2; the half-disappearance time from the patient's circulation was 15 minutes. Normal range (shown hatched) 60 to 140 minutes. The disappearance rate was not exactly exponential.

B. Utilization of Fe\textsuperscript{59} for hemoglobin synthesis. The maximum utilization was 66 per cent. In the following days the percentage decreased to 33. Assuming there was no reutilization of radioactive iron, 50 per cent of the tagged erythrocytes were destroyed in 5 days. The radioactive iron was injected 6 days after completing a course of intramuscular injections of 2 Gm. iron in total.

Investigation of hemolysis and determination of the red cell life span with the aid of radioactive chromium (Cr\textsuperscript{51}).—The osmotic fragility curves of fresh red cells were normal in both patients. After 24 hours' incubation at 37 C., some of the red cells showed a slightly increased resistance. In both cases the plasma hemoglobin concentration was below 5 mg./100 ml. The direct and indirect antiglobulin reactions were negative, and other methods also failed to reveal abnormal red cell antibodies in the blood. In patient 1, the fecal excretion of urobilinogen was quantitatively determined during 4 periods of 4 days each. The average daily excretion values were: 27, 37, 51 and 105 mg. Only the last mentioned value resulted in a hemolytic index which was high.

The life span of the patient's red cells was twice determined after labeling with radioactive chromium. During oral iron therapy the Cr\textsuperscript{51} half-value time was shortened to 14 days. On the second occasion the patient was more or less balanced without iron therapy; the half-value time was even shorter:
Fig. 9.—A, Radioactivity in blood following injection of the first patient's own erythrocytes labeled with Cr\textsuperscript{51}. T \(1/2\) Cr\textsuperscript{51} = 13 and 14 days.

B, Surface radioactivity over spleen and liver shown as a percentage of that over the precordium. The increase in splenic activity, relative to that over the precordium, from zero time to the t \(1/2\) Cr\textsuperscript{51} (13 days) was low: 8 per cent (normal range under 60 per cent; “index of sequestration,” Jandl et al.\textsuperscript{19}). The corresponding increase in liver activity was 16 per cent (within normal limits).

Fig. 10.—A, Radioactivity in blood following injection of the second patient's own erythrocytes labeled with Cr\textsuperscript{51}. T \(1/2\) Cr\textsuperscript{51} = 18 days. On a second occasion, the life span of the erythrocytes was very much more reduced, t \(1/2\) Cr\textsuperscript{51} = 5 1/2 days. The last determination was made shortly after intramuscular administration of iron (figs. 4 and 8). Abnormal hemolysis at that moment caused a very rapid decrease in hemoglobin concentration.

B, Surface radioactivity over spleen and liver shown as a percentage of that over the precordium. During the first 10 days, there was an increase in the splenic activity, relative to that over the precordium. From zero time to the t \(1/2\) Cr\textsuperscript{51} (18 days), however, no increase was demonstrable. Liver activity remained about the same during the first 10 days. Thereafter the Cr\textsuperscript{51} activity over the liver decreased to a low level.

13 days (fig. 9). Determination of surface radioactivity over the spleen showed no increase in the indexes of sequestration according to Jandl.

In patient 2 the fecal urobilinogen excretion was determined during 5 periods of 4 days each. The average daily excretion without iron therapy was 57 and 99 mg. The hemolytic index was high on the last occasion. The daily excretions during iron therapy were of much lower average value: 20, 30 and 36 mg.
The life span of the patient's red cells was twice determined after labeling with Cr$^{51}$. On the first occasion the half-value time was reduced to 18 days (fig. 10: I). Patient was then given oral iron therapy. Surface determinations first revealed increased radioactivity over the spleen, followed by a decrease, so that no lasting abnormal red cell sequestration in the spleen can be assumed. On the second occasion the red cell life span was determined during a hemolytic crisis, immediately after a course of parenteral iron therapy at the end of April 1959 (fig. 10: II). The life span of the labeled red cells was then very short (Cr$^{51}$ t $\frac{1}{2}$: 5½ days!).

**Discussion**

In these two patients, the severe anemia improved after oral or parenteral iron administration. The Hb concentration, however, showed a suboptimal rise. Transfused normal red cells disappeared from the circulation at an accelerated rate (fig. 4), indicating an extracorpuscular hemolytic defect. After administration of Fe$^{59}$, incorporation into the hemoglobin in the bone marrow was completed within a few days. These labeled red cells, therefore, were of rather uniform age. Normally the maximum utilization percentage of Fe$^{59}$ in the red cells remains at about the same level for 110 to 120 days.$^{12}$ During iron therapy, the maximum utilization percentage of intravenously injected Fe$^{59}$ in red cells of normal subjects remains at about the same level.$^{12}$ In our patients the utilization percentage of the red cells during treatment with medicinal iron showed a rapid decrease. Abnormal erythrolysis was revealed. The rapid decrease in the Fe$^{59}$ circulating red cells was compatible with hemolysis influenced by extracorpuscular factors.

Red cell antibodies were not demonstrable; no abnormal sequestration of red cells labeled with Cr$^{51}$ took place in the spleen. There was no jaundice, hemoglobinemia or hemoglobinuria. The pathogenesis of this abnormal hemolysis remains unexplained. The production of red cells and hemoglobin in the bone marrow was insufficient to afford compensation for the hemolysis, and severe anemia resulted.

The second patient showed marked reticulocytosis whenever the Hb concentration was low (fig. 4). Blood transfusions caused an increase in Hb concentration from 5.8 to 11.6 Gm./100 ml. At the same time the reticulocyte count fell to almost zero. Birkhell, Maloney and Levenson$^{8}$ likewise saw a near-arrest of erythropoiesis (in normal subjects) whenever the red cell volume was raised by more than 40 per cent with the aid of blood transfusions. The anemia in patient 2 improved during oral iron therapy; at the same time the reticulocyte count fell, as did the quantitatively determined fecal urobilinogen excretion. The pathologic breakdown of red cells appeared to be reduced by iron therapy. During treatment by intramuscular injections of iron (fig. 4), the reticulocyte count showed an initial rise to 14 per cent, followed by a decrease; the Hb concentration meanwhile increased from 6 to 10.6 Gm./100 ml. This increase was followed by fulminating hemolysis, the Hb falling within 2 weeks from 10.6 to 3.7 Gm./100 ml.; marked reticulocytosis (up to 16.6 per cent) recurred. During this hemolytic period the red cell life
span was determined \( \left( \text{Cr}^{51} t^{1/2} : 5 \frac{1}{2} \text{ days} \right) \); this value corresponds to a true life-span of some 10 days. During the same hemolytic period the utilization percentage of Fe\(^{59}\) in the red cells decreased from 66 to 33 in 5 days (fig. 8); this also corresponds to a red cell life span of about 10 days.

Why did the serum iron level remain so low (table 3) during the steady state and also during oral iron therapy and after parenteral administration, despite the presence of marked hemolysis? The serum iron level is chiefly dependent—particularly in patients with signs of iron deficiency—on the quantity of iron liberated in the course of red cell breakdown. Is the iron liberated during red cell breakdown made available to an insufficient extent for reutilization? This would seem to be suggested by the fact that—under iron therapy—Fe\(^{59}\) incorporated in the red cells rapidly decreased to very low values, and that radioactive iron was no longer demonstrable in the red cells one year after administration of Fe\(^{59}\) intravenously (in both patients). The iron liberated in red cell breakdown is likely to enter the reticuloendothelial system of liver, spleen and bone marrow, incorporated in ferritin. Vitamin C and reduced glutathione (G.S.H.) are believed to play a role in the release of iron from ferritin (Lockhead and Goldberg). The blood vitamin C concentration in our patients was normal. The G.S.H. concentration could not be determined.

The quantity of iron-binding beta globulin in the serum was always sufficient in both patients. After oral or parenteral iron therapy the beta-1 globulin was also found quite capable of binding the iron supplied. The transferrin showed no immunoelectrophoretic anomalies. Investigations based on exact localization of the Fe\(^{59}\) liberated in red cell breakdown might throw some light on these aspects. It seems possible that insufficient iron is supplied to the bone marrow for reutilization, and that this results in insufficient hemoglobinopoiesis.

Is abnormal hemolysis rare in patients with iron deficiency anemia? Rash, Cotton, Gripps and Harris have reported that the life-span of the patient's red cells, labeled with Cr\(^{51}\), can be shortened in babies with severe iron deficiency anemia. Pollycov formed the conclusion that the red cell life span may be shortened. In order to find our own answer to this question, we made an investigation in 6 other patients with hypochromic anemia and iron deficiency. Five of these were women aged 18 to 44 years. The sixth was a man aged 68, who had undergone a gastrectomy five years previously. These patients showed a suboptimal response to iron administration; no blood loss was involved. Their anemia was less severe than that in patients 1 and 2.

In the 5 women the life span of the patients' red cells was found to be shortened (Cr\(^{51}\) t \( \frac{1}{2} \): 18 to 22 1/2 days; fig 11). The impression is gained that abnormal hemolysis is more frequent in patients with iron deficiency anemia. The extent of this greater frequency can only be estimated on the basis of a larger material. The course of the anemia suggests that the iron deficiency anemia is probably the primary condition, and the abnormal hemolysis a secondary development.

Anemia of such severity as to endanger life—as encountered in our first
Fig. 11.—Radioactivity in blood following autotransfusion of erythrocytes labeled with Cr$^{51}$ in 6 patients with iron deficiency anemia. The life span of the red cells was found reduced in 5 of these patients with $t_{1/2}$ Cr$^{51}$ of 18 to 22$^{1/2}$ days. Mean normal $t_{1/2}$ Cr$^{51} = 28.3 \pm 2.3$ days (shown hatched; Verloop et al.$^{32}$).

two patients—would seem to be rare. Yet this severe anemia may well be a variety of the condition which, to a less severe degree, is not uncommonly seen in patients with iron deficiency anemia.

**SUMMARY**

A report is presented on two women suffering from severe iron deficiency anemia, concomitant with abnormal hemolysis. The life span of the patients' red cells was shortened; transfused normal red cells were more rapidly broken down. Extracorpuscular factors seem to be responsible for the pathologic hemolysis. There was no pathologic sequestration of red cells labeled with Cr$^{51}$ in the spleen.

After incorporation of radioactive iron into the red cells, the utilization percentage of Fe$^{59}$ under iron therapy fell to about 15 per cent within a few weeks. This, too, indicates that the pathologic hemolysis was to be ascribed to extracorpuscular factors. The Fe$^{59}$ was apparently not sufficiently reutilized. The constantly decreased serum iron concentration might also indicate a disturbance in the reutilization of iron liberated during red cell breakdown.

Six other patients with less severe iron deficiency anemia and an insufficient response to iron therapy were examined in addition. In 5 of these patients, the life span of red cells labeled with radioactive chromium was found shorter than normal. An insufficient response to iron therapy in patients with chronic iron deficiency anemia may be ascribable, in some instances, to concomitant pathologic hemolysis.
**FE59 STUDIES IN IRON DEFICIENCY ANEMIA WITH ABNORMAL HEMOLYSIS**

**SUMMARIO IN INTERLINGUA**

Es presentate un reporto de duo feminas sufrante de sever anemia a carentia de ferro in concomitantia con un anormalitate hemolytic. Le longevitate del erythrocytos in iste patientes eseva reducite, e transfusionate erythrocytos normal eseva decomponite plus rapidemente. Il pareva que factores extracorpuscular eseva responsabile pro le hemolyse pathologic. Un studio con erythrocytos marcate con Cr51 indicava nulle sequestration pathologic de ille cellulas in le splen.

Post le incorporation de ferro radioactive in le erythrocytos, le procentage del utilisation de Fe59 providite per le therapia a ferro descendeva a circa 15 pro cento intra alia septimana. Etiam iste constatation suggere que le hemolyse pathologic eseva ascribibile a factores extracorpuscular. Il pareva que le Fe59 non esseva re-utilisate sufficientemente. Le constantemente reducite concentration de ferro in le sero pare etiam apte a indicar un disturbation in le re-utilisation de ferro liberate durante le decomposition erythrocytic.

In plus, sex patientes con grados minus sever de anemia a carentia de ferro e un insufficiente responsa al therapia a ferro esseva examinate. In 5 de iste patientes, le longevitate de erythrocytos marcate con chromo radioactive se monstrava infra le norma. In certe casos le insufficiente responsa al therapia a ferro in patientes con chronic anemia a carentia de ferro es possibilemente ascribibile al presentia concomitante de un hemolyse pathologic.

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Radioactive Iron Studies in Patients with Iron Deficiency Anemia with Concurrent Abnormal Hemolysis

M. C. VERLOOP, M. VAN DER WOLK and A. J. HEIER