Studies of Plasma Fe\textsuperscript{56} Disappearance—A Manifestation of Ineffective Erythropoiesis and of Hemolysis\textsuperscript{*}

By Norman R. Gevirtz, Louis R. Wasserman, Lena Sharney and Dina Tendler

INEFFECTIVE ERYTHROPOIESIS, i.e., the intramedullary destruction of maturing red cells, has been postulated to explain the discrepancy between a relatively reduced peripheral red cell turnover in the presence of a normal or exaggerated erythroid marrow.\textsuperscript{1,2} This concept explains the findings in pernicious anemia and in thalassemia of increased total stercobilin excretion,\textsuperscript{3} of early tagged stercobilin peaks\textsuperscript{4,8} and of increased plasma iron with reduced red cell iron turnovers.\textsuperscript{1,2,9,10} In pernicious anemia, total stercobilin excretion has long been known to be greater than that accountable only by heme degradation from circulating effete red cells.\textsuperscript{3} After administration of isotopically labeled glycine to patients with pernicious anemia or thalassemia, a large quantity of tagged stercobilin is rapidly excreted before the appearance of significant label in the circulating red cells.\textsuperscript{6,8} To a much lesser degree, this early label of stercobilin (an early pigment “peak”) is noted in normal subjects and has been estimated to account for 10 to 20 per cent of normal total heme turnover.\textsuperscript{4,5} This peak is attributed to the “normal” or “physiologic” ineffective erythropoiesis. With the recent demonstration of bilirubin formation via nonhemoglobin pathways,\textsuperscript{11} the extent of this “normal” ineffective erythropoiesis may require a downward modification.

The concept of ineffective erythropoiesis, in which the economy of red cells, pigment and iron are all affected, is widely accepted.\textsuperscript{12-14} However, the data underlying this explanation may be interpreted on other bases. For example, the hemoglobin lost with the physiologic extrusion of the nucleus of the normoblast may cause the early pigment peaks.\textsuperscript{15} Here, iron and pigment (but not cell) economy are affected, and ineffective erythropoiesis is not implicated. The results of our studies in patients with refractory anemia with hypoplastic bone marrow suggest that ineffective erythropoiesis may affect cell economy alone, and not, detectably, pigment or iron economy. Furthermore, as has been previously noted in thalassemia,\textsuperscript{10} iron itself may be ineffectively incorporated into red cells to a greater degree than can be accounted for by pigment or cell economy.
STUDIES OF PLASMA Fe$^{59}$ DISAPPEARANCE

Our ferrokinetic observations of a relative or absolute increase in plasma radioactivity after the first day of the study may be interpreted as another manifestation of ineffective erythropoiesis on plasma radioiron behavior.

MATERIALS AND METHODS

The study subjects were as indicated in table 1 and also a group of normal controls. Autologous plasma was incubated with Fe$^{59}$, an aliquot containing 10 to 20 µc. was injected intravenously and blood sampled serially thereafter.¹⁶,¹⁷,¹⁸ (Donor plasma was used for patient M. M. because of the low latent iron binding capacity of her plasma). Two ml. of plasma were assayed for radioactivity during the initial phase of each study, and subsequently when the plasma radioactivity decreased, 4 or 5 ml. were used. Radioactivity was detected in a 3-inch well-type NaI crystal with an automatic sample changer.¹ The upper and lower discriminators of the spectrometer were adjusted to approximately 950 to 1350 Kev, to detect the two photo peaks of Fe$^{59}$. Although this setting reduced the counting efficiency to about 15 per cent, it gave the best statistics for samples with low radioactivity, and a background of 31 to 33 counts per minute. The initial plasma sample contained 2100 to 8000 CPM per 2 ml. Samples and backgrounds were alternately counted, depending upon the radioactivity for 20,000 counts on in intervals of 50 to 100 minutes for totals of 200 to 800 minutes each. Correction was made for physical decay and geometry. Standard deviation of the net count rate was determined according to Quimby and Feitelberg.¹⁹

RESULTS

The plasma Fe$^{59}$ disappearance data in M. M., a patient with congenital red cell aplasia (fig. 1) shows a prolonged initial half-time of disappearance, and a smooth, continually decreasing plasma radioactivity curve. Two other patients with refractory anemia, both with hypoplastic marrows, F. B. and S. M. (table 1) had plasma radioiron data almost identical with M. M. Radioiron incorporation into red cells in these three patients was low (table 1).

Plasma radioiron data in a normal volunteer (fig. 2) reveals a deviation from the smooth, continually decreasing plasma Fe$^{59}$ curve seen in aplastic and hypoplastic anemia. After the second day there is a transient "elevation" in plasma activity, signifying a feedback of radioiron, with a subsequent return to what might be called "baseline" activity (i.e., the projected disappearance curve) by the seventh day. This "elevation" was detected in 6 normal subjects and in a patient with polycythemia associated with hepatoma. In a seventh normal subject the "elevation" is noted only on day 2 and 3 (fig. 3).

In G. W., a patient with refractory anemia and a hyperplastic (erythroid) marrow, there is a rapid initial disappearance of radioiron from the plasma (T½ of 36 minutes) and a subsequent "elevation" which may be of longer duration than in the normal subjects (fig. 4).

An accentuated feedback of radioiron, which causes a marked "elevation" in plasma radioactivity to appear after the first day, was observed in the

¹Autowell Spectroscaler III, Picker Nuclear Corp., White Plains, N. Y.
¹¹This is not to be confused with approximations to experimental plasma Fe$^{59}$ data by three exponential functions.²⁰
Table 1.—Clinical and Laboratory Data on Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, Sex</th>
<th>Diagnosis</th>
<th>Marrow</th>
<th>Plasma Fe(^{59}) Disappearance Initial-T(^{1/2})</th>
<th>Maximum % Fe(^{59}) Incorporation into Red Cells</th>
<th>Fe/TIBC ug./100 ml. Plasma</th>
<th>HCT/Retics</th>
<th>Transfusion Requirement</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. M.</td>
<td>60, M</td>
<td>Refractory anemia (Pancytopenia)</td>
<td>Hypocellular. Relative increase basophilic normoblasts</td>
<td>397 min. 21% (Day 26)</td>
<td>298/305* 17-27/0.5-1.7</td>
<td>2-3 units/mo.</td>
<td>Normal haptoglobins (124)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. B.</td>
<td>64, F</td>
<td>Refractory anemia (Pancytopenia)</td>
<td>Hypocellular. Relative increase basophilic normoblasts</td>
<td>230 min. 32% (Day 14)</td>
<td>247/306 27-37/0.9-3.7</td>
<td>2 units/mo.</td>
<td>Cr(^{51})-red cell T(^{1/2}) = 21 days; Fe(^{59})-labeled RBC (autologous) T(^{1/2}) = 70 days. Fecal urobilinogen: 235 mg./24 hours. Repeat: 107 mg./24 hours. Remission, 10 months after diagnosis made, while on androgens and ACTH therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. M.</td>
<td>11, F</td>
<td>Congenital hypoplastic anemia</td>
<td>Absent RBC precursors. Normal WBC and platelet series</td>
<td>275 min. 22% (Day 20)</td>
<td>225/232 17/0</td>
<td>0.5-1.0 unit/mo.</td>
<td>No response to many modalities of therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. W.</td>
<td>61, M</td>
<td>Refractory anemia (and leukopenia)</td>
<td>Hypercellular with combined megaloblastosis and normoblastosis</td>
<td>36 min. 54% (Day 22)</td>
<td>143/217 25/2.3</td>
<td>None</td>
<td>No response to B(_12), B(_6), and other vitamins. No response to steroids, androgens, etc. Cr(^{51}) RBC T(^{1/2}) = 20 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. W.</td>
<td>56, M</td>
<td>Polycythemia and carcinoma of liver (primary? metastatic?)</td>
<td>Erythroid hyperplasia</td>
<td>21 min. 100% (Day 5)</td>
<td>75/390 63/2.7</td>
<td>None</td>
<td>RBC mass 69 ml./Kg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. B.</td>
<td>65, M</td>
<td>Thalassemia minor with secondary hemochromatosis</td>
<td>Erythroid hyperplasia</td>
<td>31 min. 42% (Day 8)</td>
<td>220/245 33/3.1</td>
<td>None</td>
<td>Hb A(_2) = 5.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. K.</td>
<td>64, M</td>
<td>Autoimmune hemolytic anemia</td>
<td>Erythroid hyperplasia</td>
<td>32 min. 72% (Day 11)</td>
<td>80/231 30/7.9</td>
<td>None</td>
<td>Coombs—negative, Cr(^{51}) RBC T(^{1/2}) = 17 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. O.</td>
<td>66, M</td>
<td>Megaloblastic anemia (Malabsorption of B(_12) and folic acid)</td>
<td>Megaloblastic</td>
<td>42 min. 55% (Day 11)</td>
<td>98/208 35/2.0</td>
<td>None</td>
<td>Responded to parenteral vitamin B(_12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J. G.</td>
<td>54, M</td>
<td>Mild cirrhosis of liver: acute alcoholic intoxication</td>
<td>Not done</td>
<td>225 min. 90% (Day 22)</td>
<td>See Table II 43/1.8</td>
<td>None</td>
<td>See text</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. K.</td>
<td>20, M</td>
<td>Encephalitis, chronic</td>
<td>Not done</td>
<td>40 min.</td>
<td>Not done</td>
<td>86/305 44/1.8</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J. L.</td>
<td>38, M</td>
<td>Essential hypertension</td>
<td>Not done</td>
<td>73 min.</td>
<td>Not done</td>
<td>100/381 44/1.6</td>
<td>None</td>
<td>BUN 19 (normal)</td>
<td></td>
</tr>
</tbody>
</table>

*Autologous plasma used for Fe\(^{59}\) study had UIBC of 70.
Fig. 1.—Plasma Fe$^{59}$ disappearance expressed as a per cent of the extrapolated t<sub>1/2</sub> value (●——●) and per cent cell Fe$^{59}$ incorporation (○——○) in M. M., a patient with red cell aplasia.

data on three patients, the first with thalassemia minor (fig. 5), the second with autoimmune hemolytic anemia and the third with mild megaloblastic anemia (hematocrit 35 per cent) due to malabsorption of vitamin B<sub>12</sub> and folic acid (fig. 6). In this latter data an additional “elevation” is seen after an injection of vitamin B<sub>12</sub> was given (fig. 6).

J. G., who had mild cirrhosis of the liver, transiently altered his iron metabolism after an alcoholic bout just prior to the study. Serum iron increased (table 2), and the initial half-time of radioiron disappearance from plasma, 225 minutes, was prolonged (fig. 7) when compared to normal. The plasma radioiron “elevation” found in this patient persisted from the second day to
Fig. 2.—Plasma Fe\(^{59}\) disappearance expressed as a per cent of the extrapolated \(t_e\) value (■ ■ ■) and per cent red cell Fe\(^{59}\) incorporation (○ ○ ○) in E. A., a normal volunteer. The different standard deviations reflect differences in the total counts accumulated for each sample (and its background). This is due, primarily, to different total counting durations.

perhaps as long as the tenth (fig. 7). Erythropoiesis also may have been temporarily altered because only 57 per cent of radioiron was incorporated into red cells by day 7 and 70 per cent by day 11.

In an attempt to better define the significance of this radioiron feedback, plasma was obtained twice daily in several subjects and a diurnal variation of plasma radioactivity was noted (fig. 8). The radioactivity of the morning specimens (8 to 10 A. M.) is higher than in the specimens obtained in the late afternoon or evening, 4 to 10 P. M., (except on day 8 for patient J. L.).

Twenty-four hours after Fe\(^{59}\) injection, the plasma radioactivity in the 6
normal subjects was 0.19 to 0.29 per cent of the extrapolated initial radioactivity. For the patients with normal or increased erythropoiesis (table 1) the twenty-four-hour values ranged from 0.07 to 0.40 per cent of the initial plasma radioactivity. (In patient J. G. (see above) this value was 2 per cent). In the 4 patients with aplastic or refractory anemia (table 1) the plasma radioactivity at twenty-four hours was 1.2 per cent (G. W.) to 7.0 per cent (M. M.) of the initial value.

**Discussion**

The transient "elevation" of plasma radioiron activity after twenty-four hours is noted only with normal or increased marrow erythroid activity (figs. 2-7).
The temporal association of this change in plasma radioiron with the increase in labeled pigment excretion suggests a cause and effect relationship. After administration of labeled amino acid, the plasma bilirubin peak attributed to early heme breakdown occurs about the fourth day, and the early labeled stercobilin excretion peak occurs about the same time. This timing, in general, is the same as for the maximal plasma Fe\textsuperscript{59} concentration. The deviation may thus be interpreted as representing the return to plasma of radioiron from those heme containing marrow cells which are destroyed as a result of ineffective erythropoiesis. This feedback may increase plasma radioiron activity for a longer time than the duration of the early pigment peak. Iron from effete red cells is redistributed among the several interconnected "pre-and posterythropoietic" pools of iron metabolism. In transit through these pools there may be a time delay before the iron reappears in the plasma. Bilirubin excretion, on the other hand, has been shown to occur quickly after its rapid formation from hemoglobin. These multiple iron pools and their temporizing effect on the return of iron to plasma may also explain the similarity of plasma radioiron curves in patients with intense hemolysis, whether predominantly due to ineffective erythropoiesis of varying etiology (figs. 5, 6) or to autoimmune hemolytic anemia.
Although ineffective erythropoiesis with its implication of destruction of maturing red cells is the most probable explanation for the "elevations" of plasma radioiron reported here, other factors may play a role. Radioiron from ferruginous granules removed from siderocytes by the spleen may augment the plasma radioactivity. Siderocyte iron may represent an excess of iron above the normal need, and from the point of view of iron economy this represents ineffective utilization of iron. Plasma radioiron may possibly be increased by the return of radioiron from the degraded hemoglobin physiologically lost with the normal extrusion of the erythroblast nucleus. Here the economy of both iron and pigment is impaired. In neither situation (fer-
Fig. 6.—Plasma Fe⁵⁹ disappearance expressed as a per cent of the extrapolated t₀ value (●—●) and per cent red cell Fe⁵⁹ incorporation (○—○) in F. O., a patient with megaloblastic anemia due to malabsorption of vitamin B₁₂ and folic acid. The different standard deviations reflect differences in the total counts accumulated for each sample (and its background). This is due, primarily, to different total counting durations.

Ruginous granule or perinuclear hemoglobin loss is there destruction of cells. Therefore, ineffective erythropoiesis—with its implication of impaired cellular economy—is not a factor.

The “elevation” of plasma radioactivity is not manifest in patients M. M. (fig. 1), S. M. and F. B. The ferrokinetic data on these 3 patients are typical for aplastic anemia. However, the marrow films of S. M. and F. B. reveal hypoplastic marrow with a relative “maturation arrest” at the basophilic normoblast stage of maturation, and not an aplasia as for M. M. (table 1). Histologic evidence of an “arrest” implies cell death at or immediately beyond the observed stage of maturation and represents true ineffective erythropoiesis. It has been shown that the normal basophilic erythroblast has half or more of the total iron content of a mature erythrocyte. The failure of this ineffective erythropoiesis to be reflected in the plasma radioiron data may be a composite of several factors: (A) The total marrow-erythroid activity is decreased; (B) the injected radioiron is diluted by the abnormally
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Table 2.—Plasma Iron and Iron Binding Capacity on J. G.

<table>
<thead>
<tr>
<th>Day of Study</th>
<th>Fe+11</th>
<th>UIBC</th>
<th>TIBC</th>
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<tbody>
<tr>
<td>-11</td>
<td>113</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0</td>
<td>365</td>
<td>21</td>
<td>386</td>
</tr>
<tr>
<td>0 + 2 hrs.</td>
<td>360</td>
<td>31</td>
<td>391</td>
</tr>
<tr>
<td>0 + 7 hrs.</td>
<td>340</td>
<td>50</td>
<td>390</td>
</tr>
<tr>
<td>1</td>
<td>340</td>
<td>114</td>
<td>454</td>
</tr>
<tr>
<td>5</td>
<td>113</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>113</td>
<td>222</td>
<td>375</td>
</tr>
<tr>
<td>9</td>
<td>97</td>
<td>218</td>
<td>315</td>
</tr>
<tr>
<td>17</td>
<td>90</td>
<td>399</td>
<td>489</td>
</tr>
<tr>
<td>22</td>
<td>155</td>
<td>263</td>
<td>418</td>
</tr>
</tbody>
</table>

For two days prior to day 0 the patient was drinking heavily. The study was begun while the patient was recovering from acute alcoholic intoxication. On days 20 and 21 the patient was drinking heavily again.

large iron content in the "transport subsystem";29 (C) there is rapid shunting of this radioiron to the liver, as much for these patients as for M. M. (as estimated from body surface scanning data). Hence little of the diluted radioiron reaches the marrow. Therefore, (D) in these 2 patients (S. M., F. B.) the small quantity of radioiron feeding back to plasma from maturing red cells catabolized in the marrow is obscured by the persistently high radioactivity in the plasma. This is not true for G. W. (fig. 4) who had a hyperplastic marrow with marked ineffective erythropoiesis, and in whom the feedback caused a distinct plasma radioiron "elevation."

The possibility that the "elevations" in plasma Fe59 activity are artifacts due to (test-tube) hemolysis of newly formed radioactive red cells must be considered. Figure 8 shows how the plasma radioactivity follows the diurnal pattern for serum iron concentration,30,31,32 and fig. 7 demonstrates the same levels of radioactivity in 2 successive specimens (day 3) and in 4 successive specimens obtained during a 3-hour period (day 5). Furthermore, plasma hemoglobin levels were not elevated. These observations minimize the possibility of an artifactual increase in plasma Fe59 due to laboratory handling of blood with radioactive red cells.

The data show that at 24 hours only about 0.2 per cent of the initial plasma radioactivity remains when there is active erythropoiesis, a finding not pre-reported. Monti, Glynn and Derr33 observed this only once: their other data showed over 1 per cent plasma radioactivity at 24 hours. Pollycove and Mortimer18 found 0.7 to 2.7 per cent of the initial radioactivity present in their normal subjects at 24 hours. The reasons for our detecting consistently lower radioactivity is not readily apparent.

**Summary**

1. Studies of the plasma radioiron disappearance data have revealed a relative "elevation" of plasma radioactivity after the second day in normal subjects, and after the first day in patients with hemolytic anemia, megaloblastic anemia, and another two patients with hyperplastic marrow. The possibility that the "elevations" in plasma Fe59 activity are artifacts due to (test-tube) hemolysis of newly formed radioactive red cells must be considered.
Fig. 7.—Plasma Fe$^{59}$ disappearance expressed as a per cent of the extrapolated $t_e$ value (●—●) and per cent red cell Fe$^{59}$ incorporation (○—○) in J. G., a patient with acute alcoholism.

blastic anemia and thalassemia. This elevation in plasma radioactivity in normal subjects may be interpreted as due to the return to plasma of radioiron from those cells that are "ineffectively" produced and are hemolyzed in the marrow. Other interpretations are also presented.

2. Dyspoieses involving ineffective red cell economy, but not detectably affecting iron economy are contrasted with disorders involving ineffective utilization of iron and of pigment alone.

3. After the first day plasma radioiron activity shows a diurnal variation similar to the diurnal variation in plasma iron concentration.

**Summario in Interlingua**

1. Datos obtenite in studios del disparition de radioferro ab le plasma reflecte un relative "elevation" del radioactivitate del plasma post le secunde die in subjectos normal, e post le prime die in patientes con anemia hemolytic, anemia megaloblastic, e thalassemia. Iste elevation del radioactivitate plasmatic in subjectos normal pote esser interpretate como un reflexion del retorno, ad le plasma, de radioferro ab le cellulas que es producite "inefficacemente" e
Fig. 8.—The diurnal variation of plasma radioiron activity. The different standard deviations reflect differences in the total counts accumulated for each sample (and its background). This is due, primarily, to different total counting durations.

que es hemolysate in le medulla. Es etiam presentate altere interpretationes possibile.

2. Dyspoieses que reflecte un inefficacia in le economia del erythrocytos, sed que non affice de manera detegibile le economia de ferro, es ponite in contrasto con disordines in que il se tracta de un inefficace utilisation de ferro e de pigmento sol.
REFERENCES


STUDIES OF PLASMA FE$^{59}$ DISAPPEARANCE


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