Erythrokinetic Studies in Severe Bone Marrow Failure of Diverse Etiology

By Norman R. Gevirtz and Nathaniel I. Berlin

Refractory anemia is defined as anemia responsive only to blood transfusion. Primary refractory anemia is distinguished from secondary refractory anemia in that there is no associated infection, renal or hepatic disease, malignancy or malnutrition.1

The etiology, clinical and laboratory findings, therapy, and prognosis of acquired aplastic anemia have been reviewed recently by Scott and co-workers2 and by Mohler and Leavell.3 Studies of iron metabolism, generally either the plasma radioiron clearance rate,2,4,9 uptake into circulating red cells,10 red cell iron turnover4 or in vivo measurement of iron transport have been reported.4 In general, these reports have been concerned with the evaluation of these methods and not with studying a given disease state. These studies have shown that the plasma radiciiron clearance $T^{1/2}$ is prolonged, with diversion of the radioiron principally to the liver and with a low uptake in circulating red cells. This paper presents the results of studies of erythropoiesis in seven patients with refractory anemia, each with different etiology or underlying disease.

Methods

The methods used in this study for the determination of the total red cell volume, plasma volume, rate and site of formation and life span of the red cells have been previously described in detail.11 The blood volume was also determined by the P32 labeled red cell method of Mollison.12 The P32 was counted by plating aliquots on lens tissue, covering with cellophane paper and wrapping around a Geiger-Mueller tube. The patients were all hospitalized on the Metabolism Service, at least during the initial phase of the study.

Case Histories and Results

Case I CC 02-05-33

This 52 year old white male was referred to the National Cancer Institute in July 1958. He stated he had enjoyed good health until the summer of 1957 when he noted the onset of fatigue and petechiae. The diagnosis of aplastic anemia was established in December 1957 from examination of peripheral blood and bone marrow aspirations. In his occupation as a printer, he had contact with benzol, carbon tetrachloride, and “freon” for seven years prior to the onset of his illness. From December 1957 through April 1958, in addition to supportive transfusions he received courses of methylprednisolone, folic acid, vitamin B12, and three doses of parenteral testosterone, all without benefit. On admission to the National Cancer Institute the patient complained of easy fatigability and spontaneous appearance of petechiae and ecchymoses. Physical examination revealed scattered ecchymoses and generalized petechiae, especially on the lower extremities. There were small shotty inguinal lymph nodes, but no palpable enlargement of the liver or spleen. The patient received 35 units of blood from December 1957 until death in August 1958. At autopsy...
Fig. 1a.—The isotopic data and transfusions on patient I.
Fig. 1b.—The peripheral blood values and transfusions on patient I.
there was aplasia of the marrow, a generalized hemorrhagic diathesis, and multiple abscesses
due to clostridia, staphylococcus and aspergillus. The spleen weighed 200 Gm. and the
liver 2,200 Gm.; both showed hemosiderosis.

The erythrokinetic studies (see figure 1a) shows prolonged half-time of plasma Fe\(^{59}\)
disappearance, uptake of Fe\(^{59}\) by the liver with negligible uptake of Fe\(^{59}\) by the
marrow and the spleen. There was minimal incorporation of Fe\(^{59}\) into the erythrocytes by 18
days. The Cr\(^{51}\) red cell apparent survival half-time was 14 days, with accumulation of Cr\(^{51}\)
in the liver and spleen.

Figure 1b shows the pancytopenia and the transfusion requirement. The reticulocyte
count, not graphed, was zero during the study.

Case II CC 02-13-27

This 71 year old white male was referred to the National Cancer Institute in September
1958. The patient stated that he was in good health until March 1957 when he noted the
onset of weakness, fatigability and an acute episode of vomiting. An anemia of unknown
etiology was discovered, and the patient required one unit of blood per month. He was given
therapeutic trials of parenteral liver, vitamin B\(_{12}\) and oral iron without benefit. In June 1958,
hepatosplenomegaly was found. Several bone marrow aspirations done during June and
July 1958 were reported normal or hypercellular. Needle biopsy of the liver performed in
August 1958 showed extramedullary hematopoiesis and established the diagnosis of myeloid
metaplasia. Physical examination upon admission revealed the spleen and liver each palpable
six cm. below the costal margins. No lymph nodes were palpable. Surgical sternal bone
marrow biopsy revealed marked hypercellularity with many megakaryocytes. Bone marrow
smear revealed a slight shift to the left of the granulocytic series. The patient was given
therapeutic trials of trimethyl colchicinic acid, adrenal cortical hormones and testosterone
with no improvement. He required about one unit of blood every second day for several
weeks prior to death in May 1959. Necropsy revealed myelofibrosis and hypoplasia of the
hematopoietic elements of the marrow with some areas of hypercellularity. The spleen
showed extra medullary hematopoiesis and the liver and spleen hemosiderosis.

The erythrokinetic studies, figure 2a, demonstrates a normal plasma iron disappearance
half-time with uptake of Fe\(^{59}\) by the spleen and liver. There is slight but definite uptake
of Fe\(^{59}\) by the sacral marrow. Fifteen per cent of the radioiron appears in erythrocytes.
There is, similarly, diminished incorporation of the C\(^{14}\) labeled glycine into hemoglobin.
The Cr\(^{51}\) survival half-time is 19 days, with accumulation of Cr\(^{51}\) in the spleen and the
liver.

Figure 2b shows the peripheral blood counts, the transfusion requirement and the
therapeutic regimens. The platelet count, initially normal, rapidly fell to low levels. There
was intermittent leukopenia. A large transfusion requirement developed when the prednisone
dosage was decreased from 200 to 100 mg. per day. This abated when the prednisone
dosage was restored to 200 mg. per day.

Case III CC 01-87-68

This 37 year old white female was referred to the National Cancer Institute in September
1957. She stated that the diagnosis of chronic myelogenous leukemia was established in
July 1957 by peripheral blood and bone marrow examination after a brief period of weakness,
fatigue and left upper quadrant fullness. She was treated with vitamin B\(_{12}\) injections
with subjective improvement. At the National Cancer Institute she was treated with Col-
cinide from September 1957 through August 1958 and with oral ferrous sulfate from
June 1958 through August 1958. These were discontinued when increasing anemia and
splenomegaly developed. Bone marrow aspiration on September 4, 1958 was compatible
with chronic myelogenous leukemia. She was then treated with x-radiation to the spleen,
trimethyl colchicinic acid, methylprednisolone and 6-mercaptopurine (fig. 3b). She died
on April 27, 1959 after having received 110 units of blood since September 28, 1958.
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the last month of life, several bone marrow examinations were indistinguishable from acute myelogenous leukemia. At necropsy the liver weighed 3,000 Gm. and the spleen 1,850 Gm.; the spleen showed hemosiderosis.

The erythrokinetic studies (fig. 3a) demonstrate an extraordinarily rapid clearance of radioidron from the plasma. The Fe$^{59}$ is taken up predominantly by the spleen, with lesser uptakes by the liver and marrow. Approximately 25 per cent of the total Fe$^{59}$ appears in the peripheral blood. The Cr$^{51}$ labeled red cell $T_{1/2}$ is 9 days with accumulation of Cr$^{51}$ in the spleen and liver. If it is assumed that the decrease in Fe$^{59}$ content/ml./RBC observed at 80 days represents the failure to re-utilize iron released from senescent cells at the time, then the life span of the patient's own red cells is approximately 80-90 days. This is in marked contrast to the Cr$^{51}$ red cell survival data.

Figure 3b shows the peripheral blood counts and therapy for the duration of the erythrokinetic study.

Case IV CC 04-89-08

This 72 year old white male was referred to the National Cancer Institute in September 1957. He gave a history of enjoying good health until July 1953 when he noted weakness and palpitations. A diagnosis of aplastic anemia was made from peripheral blood and bone marrow aspirations. In 1954 two marrow aspirations were acellular. A third (surgical) marrow biopsy revealed eosinophilic and erythroid hyperplasia. Therapy with cortisone, vitamin B$_{12}$, pyridoxine, folic acid, citrovorum factor, yeast nucleic acid and liver extract were unsuccessful. Intravenous and retrograde pyelography showed a mass in the lower pole of the left kidney, for which a left nephrectomy and splenectomy were done on March 21, 1956. A clear cell carcinoma of the kidney was found. The spleen weighed 330 Gm. and was hemosiderotic. Postoperatively the only improvement was in the leucocyte and platelet count for a period of one month. Physical examination on admission showed only previous surgical incisions. Bone marrow aspirations (September 1957, and October 1957) demonstrated active hematopoiesis with evidence of probably abnormal maturation of erythroid series. On November 8, the patient underwent a cholecystostomy for an acute cholecystitis. He recovered satisfactorily and returned to the referring hospital for further care. He died on January 5, 1958. Autopsy revealed metastatic renal cell carcinoma and hyperplasia of the bone marrow chiefly due to cells of the erythroid series. The liver weighed 2,050 Gm. and contained extensive deposits of hemosiderin.

The erythrokinetic studies (fig. 4a) shows a normal half-time for the plasma Fe$^{59}$ disappearance with incorporation of the iron in the liver and marrow. A maximum of 10 per cent of the injected Fe$^{59}$ appears in the circulating erythrocytes. There is a late and unexplained rise in the counting due to Fe$^{59}$ over the liver. The incorporation of C$^{14}$ labeled glycine into hemoglobin was low. The Cr$^{51}$ half-time survival was 16 days with minimal accumulation of Cr$^{51}$ in the liver.

Figure 4b shows the peripheral blood counts. There was considerable variation, in the platelet, white cell and reticulocyte counts. The large transfusion requirement is demonstrated.

The multiple transfusions required for the cholecystostomy on November 8 precluded study of the incorporation of C$^{14}$ labeled glycine into hemoglobin after day 60. The available data indicate that the patient's own red cells had a life span greater than 60 days.

Case V CC 02-72-77

This 29 year old white female was referred to the National Cancer Institute in November 1959. She stated that she was in good health until December 1952 when she experienced excessive fatigue and weakness during her second pregnancy. Her physician stated that the blood count was low and prescribed dextro-amphetamine-sulfate-amobarbital tablets. She took three or four tablets daily from December 1952 until October 1954. In June 1953 she delivered a normal full term child. A breast infection on the 11th postpartum day was treated with oxytetracycline.
Fig. 2a.—The isotopic data and transfusions on patient II.
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Fig. 2b.—The peripheral blood values, transfusions and therapy on patient II. T.M.C.A. = Trimethylcolchicine acid.

After delivery she continued to experience weakness, fatigue and palpitations. She had a bruising tendency during the summer of 1954. A severe anemia (red blood count less than $1 \times 10^6$/cu.mm.) was noted in October 1954. Laboratory studies including bone marrow examination were not diagnostic. From October 1954 until admission to the National Cancer Institute, she received about three units of blood per month for a total of approximately 200 units. Therapeutic trials with many vitamin preparations, "hematinics," vitamin $B_12$, liver extract, busulfan and cortisone were without benefit. Bone marrow aspirations done after 1957 showed at first suggestive, later more definite, evidence of myelofibrosis. In 1959, hepatomegaly, transient splenomegaly and hemosiderosis of the
Fig. 3a.—The isotopic data and transfusions on patient III.
Fig. 3b.—The peripheral blood values, transfusions and therapy on patient III. T.M.C.A. = Trimethylcolchicinic acid.
Fig. 4a.—The isotopic data on patient IV.
Fig. 4b.—The peripheral blood values and transfusions on patient IV.
skin were noted. Surgical bone marrow biopsy in November 1959 showed myelofibrosis and marked hypoplasia. While on testosterone enanthate, 600 mg. I.M. weekly, the liver enlarged. On March 15, 1960 she developed chills and spiking fever. Several days later, signs and symptoms of an acute "surgical abdomen" became manifest. On March 24, 1960, exploratory laparotomy revealed a massively enlarged liver extending into the left gutter and perisplenitis in a modestly enlarged organ (weight estimated at 300 Gm.). Splenectomy was thought to be hazardous, and was not done.

Fever, numerous respiratory infections, a rising white blood count with a large proportion of unidentifiable cells and the need for frequent transfusions characterized her course until March 1961 when a persistent upper respiratory infection occurred.

The patient developed congestive heart failure which did not respond to therapy. At autopsy the spleen weighed 650 Gm. and the liver 4,750 Gm. Both the spleen and liver showed extensive hemosiderin deposits and a cellular infiltrate compatible with leukemia. The bone marrow—in contrast to previous findings of either myelofibrosis or hypoplasia with several occasions when no marrow particle was obtained—was hyperplastic. There were a few normoblasts scattered throughout the leukemia-like infiltrate. A diagnosis of hemochromatosis in addition to transfusion hemosiderosis can be made on the basis of iron deposits in the epithelial cells of intrahepatic bile ducts, extensive deposit of pigment in the pancreas and cirrhotic changes in the liver.

The erythrokinetic data (fig. 5a) shows slow clearance of radioiron from the plasma, with uptake by the liver, and to a lesser extent by the spleen and marrow. There is no demonstrable erythropoietic activity as evaluated by the lack of uptake of Fe59 or of C14 in the peripheral red cells. The Cr51 apparent survival half-time is 20 days.

A second study (not illustrated) started after 6 weeks of androgen therapy showed even further prolongation of the plasma Fe59 half-time disappearance to 5 hours, and as before, no utilization of the Fe59 for red cell synthesis.

The peripheral blood counts are graphed in figure 5b and the transfusion requirement demonstrated. An improvement in the platelet count occurred just prior to, and immediately after surgery. Reticulocytes, not graphed, were always absent.

Case VI CC 02-11-12

This 74 year old white female was referred to the National Cancer Institute in August 1958. She stated that she was in good health until December 1956 when she noted easy fatiguability and neck swelling. A biopsy of a cervical lymph node was interpreted as chronic lymphatic leukemia or lymphosarcoma. Peripheral blood counts are not available for this period. She allowed only local x-ray therapy to her back for relief of pain. Hepatosplenomegaly and increasing anemia developed by April 1958. This was treated with transfusions and chlorambucil. Immediately prior to chlorambucil therapy the peripheral leukocyte count was 12,000/mm.3 with 34 per cent polymorphonuclear cells and 52 per cent lymphocytes. Increasing anemia and leukopenia were noted when she was referred to the National Cancer Institute. Bone marrow aspiration, September 1958, revealed a hypercellular marrow with 80 per cent erythroid series and, at most, 25 per cent lymphoid series. When she was transfused to 15 Gm. per cent hemoglobin her marrow changed to one containing 90 per cent lymphocytes. She died on March 1, 1959 after a course complicated by hepatitis, pneumonitis and cerebral vascular accidents. At autopsy the liver was hemosiderotic and weighed 1,450 Gm. and the spleen weighed 160 Gm.

The erythrokinetic studies (fig. 6a) shows that clearance from the plasma of Fe59 is slightly prolonged. The Fe59 is largely taken up by the liver, and to a lesser extent by the spleen and marrow. A negligible amount of Fe59 is incorporated into erythrocytes. The Cr51 apparent survival half-time was 15 days with evidence of accumulation of Cr51 by the liver and spleen.

The peripheral blood counts for the period of the erythrokinetic study is shown in figure 6b. The reticulocyte count, not graphed, was 0.1 per cent or less.
Case VII CC 02-84-98

This 28 year old white male was referred to the National Cancer Institute in February 1960. He stated that he was in good health until December 1959, when he noticed petechiae and purpura on his legs. A diagnosis of aplastic anemia was made from examination of peripheral blood and bone marrow aspirations and biopsy. Past history includes contact with carbon tetrachloride, acetylene and arc welding fumes, insecticides with nicotine and DDT, paint solvents (none known to have benzene) and fumes from various fires in his capacity as a fireman. Except for skin and retinal hemorrhages, physical examination was not remarkable. The liver and spleen were not palpably enlarged. Therapy with adrenal steroids from January 13, 1960 through May 12, 1960 gave no obvious improvement. Large doses of androgen were of questionable efficacy. The patient was given blood transfusions when indicated and fresh plasma for platelets when episodes of gastrointestinal and retinal hemorrhage occurred. He died on June 13, 1960 of a massive intracerebral hemorrhage. The bone marrow was hypoplastic. The spleen weighed 110 Gm., the liver 2,060 Gm.; there was marked hemosiderosis of the liver and pancreas and also hemosiderin deposits in the spleen.

The erythrokinetic studies (fig. 7a) show a very prolonged clearance of Fe$^{59}$ from the plasma. The liver, almost exclusively, stores the radioiron. Approximately 8 per cent of the Fe$^{59}$ appears in the peripheral red blood cells. Similarly, there is minimal incorporation of C$^{14}$ from labeled glycine in the red cells. There is a suggestive increase in the C$^{14}$ specific activity starting at about 60 days. This increase is obscured by the transfused blood that lowers the specific activity by dilution.

The second erythrokinetic study started on May 11, 1960 and after 10 weeks of androgen therapy shows a slight decrease of the plasma Fe$^{59}$ half-time disappearance. The radioiron is again taken up principally by the liver, but somewhat more than before is take up by the marrow. About 16 per cent of the Fe$^{59}$ given is utilized for erythrocyte production. In agreement with this is the slightly increased incorporation of glycine 2-C$^{14}$ into erythrocytes. A Cr$^{51}$ labeled red cell study, started on May 28, 1960, showed an apparent survival half-time of 12 days. There was no accumulation of Cr$^{51}$ in the spleen or the liver.

Figure 7b shows the peripheral blood counts and therapy. Pancytopenia, high transfusion requirement, including platelet transfusions (fresh plasma) and peripheral response to these transfusions are graphed. An increase in the reticulocyte and per cent polymorphonuclear count in the peripheral blood occurred after androgen therapy. Prednisone therapy did not seem to alter the peripheral blood counts, although there is a suggestive increase in transfusion requirement after cessation of prednisone therapy.

**DISCUSSION**

These seven patients had in common one feature—failure to synthesize red cells. However, they were a heterogeneous group from several standpoints—bone marrow cytology and histology, underlying or associated specific disease state and stage of disease.

These patients are grouped together as Bomford and Rhoads' did for convenience in reporting because they represent a single end result of various abnormal processes and because from the current therapeutic standpoint they are alike. The patient with little or no cytologic evidence of erythropoiesis in the marrow—or elsewhere—has a partial explanation for the anemia. Yet the processes producing this anatomical loss of cells are not known. On the other hand, it is likely that in the patients with anatomical evidence of erythropoiesis there are other abnormal processes operative, which lead to the same end result—failure to deliver red cells to the peripheral blood.
Fig. 5a.—The isotopic data and transfusions on patient V.
Fig. 5b.—The peripheral blood values, transfusions and therapy on patient V.

It is convenient, at present, to categorize such patients as having an anemia responsive only to blood transfusion and thus to be refractory to other methods of therapy. In the future, it is likely that these types of patients will not be grouped together, and different specific mechanisms uncovered to explain the results, permitting a better classification.

**Blood Volume**

All seven patients had a total red cell volume 24 cc./Kg. or less and should be considered anemic. The plasma volume was elevated above the normal range for this method, but the blood volume tended to remain within normal limits (table 1).
Fig. 6a.—The isotopic data and transfusions on patient VI.
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Fig. 6b.—The peripheral blood values and transfusions on patient VI.

Iron Metabolism Studies

The serum iron (with the exception of patient III) was elevated and the iron binding protein saturated.

The T₁/₂ for plasma Fe⁵⁹ clearance was within the normal limits in two
patients (II and IV), was increased in four patients and markedly decreased in one patient (III). In both the dog and man increased body iron stores decrease the incorporation of intravenously administered Fe⁵⁹ into circulating erythrocytes,¹⁶ and prolong the plasma Fe⁵⁹ clearance T₁/₂.⁶ Patients I, V, and VI who showed prolonged plasma Fe⁵⁹ clearance T₁/₂'s had sufficient transfusions prior to these studies to increase significantly the body iron stores. This was not so in patient VII who had the slowest plasma radioiron clearance. Furthermore, in an experimentally produced decrease in erythropoiesis, the T₁/₂ for the plasma Fe⁵⁹ clearance was increased comparable to that observed in these patients.¹³ In the absence of an erythropoietically active bone marrow the observed prolonged clearance T₁/₂ of iron from the plasma is presumably
Fig. 7b.—The peripheral blood values, transfusions and therapy on patient VII.
<table>
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<tr>
<th>Patient</th>
<th>Age at time of study and sex</th>
<th>Diagnosis</th>
<th>Date of study</th>
<th>Method</th>
<th>Blood volume</th>
<th>Plasma iron turnover</th>
<th>RBC iron turnover</th>
<th>Lifespan</th>
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<td>I</td>
<td>32-M</td>
<td>Aplastic anemia toxic (benzol)</td>
<td>7/21/58</td>
<td>Cr&lt;sup&gt;51&lt;/sup&gt;</td>
<td>12.2 63.8</td>
<td>226 0</td>
<td>0.82 0.016</td>
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<td>II</td>
<td>71-M</td>
<td>Agranocytic myeloid metaplasia</td>
<td>11/3/58</td>
<td>Cr&lt;sup&gt;51&lt;/sup&gt;</td>
<td>24.3 61.8</td>
<td>201 0</td>
<td>1.30 0.13</td>
<td>80-90 days</td>
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<td>III</td>
<td>37-F</td>
<td>Chronic myelogenous leukemia</td>
<td>9/10/58</td>
<td>Cr&lt;sup&gt;51&lt;/sup&gt;</td>
<td>16.6 47.5</td>
<td>36 not done</td>
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<td>90 days</td>
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<td>IV</td>
<td>72-M</td>
<td>Aplastic anemia idiopathic (hyperplastic marrow)</td>
<td>9/9/57</td>
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<td>15.4 54.1</td>
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<td>V</td>
<td>29-F</td>
<td>Myeloblastosis</td>
<td>11/18/59</td>
<td>Cr&lt;sup&gt;51&lt;/sup&gt;</td>
<td>15.2 44.4</td>
<td>202 0</td>
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<td>VIII</td>
<td>28-M</td>
<td>Aplastic anemia idiopathic (? toxic)</td>
<td>2/25/60</td>
<td>Fe&lt;sup&gt;59&lt;/sup&gt;</td>
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<td>0.29 0.023</td>
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<td>5/11/60</td>
<td>Fe&lt;sup&gt;59&lt;/sup&gt;</td>
<td>19.2 64.2</td>
<td>192 8</td>
<td>0.32 0.048</td>
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<td></td>
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<td>5/28/60</td>
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*Calculated from the plasma volume as determined by extrapolation of plasma Fe<sup>59</sup> disappearance curve and the venous hematocrit.
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Table 2.—Serum and Unsaturated Iron Binding Capacity in Patient III

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<th>Date</th>
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<td>9/12/57</td>
<td>62</td>
<td>120</td>
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<td>6/20/58</td>
<td>306</td>
<td>60</td>
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<tr>
<td>6/20/58</td>
<td>oral iron started</td>
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</tr>
<tr>
<td>8/22/58</td>
<td>59</td>
<td>80</td>
</tr>
<tr>
<td>9/2/58</td>
<td>oral iron discontinued</td>
<td></td>
</tr>
<tr>
<td>9/3/58</td>
<td>38</td>
<td>94</td>
</tr>
<tr>
<td>9/10/58</td>
<td>36</td>
<td>(not done)</td>
</tr>
<tr>
<td>9/25/58</td>
<td>first transfusion given</td>
<td></td>
</tr>
<tr>
<td>10/3/58</td>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td>11/6/58</td>
<td>97</td>
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<td>1/16/59</td>
<td>36</td>
<td>32</td>
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due to the normally inefficient clearance of plasma iron by the liver and spleen.

The unusually rapid clearance of radioiron from the plasma in patient III is unexplained. This patient had not received transfusions prior to these studies, but had been treated with oral iron preparations for several months. Fe59 given as a salt, unbound to siderophilin prior to injection, has a normal clearance half-time when given to patients with nonhematologic diseases and normal subjects.6'14 The rapid clearance of Fe59 from the plasma in this patient is probably a valid measure of the plasma iron turnover since the observation was documented by five measurements during the first hour, the donor plasma lot used to prepare the Fe59-siderophilin complex had previously and subsequently been used with satisfactory results in other patients, and this patient's iron binding protein was not saturated at this time. The low serum iron observed in this patient is undoubtedly due to the rapid clearance of siderophilin bound iron from the plasma. Additional evidence of unusual iron metabolism in this patient is seen in table 2.

Previous studies in aplastic anemia have demonstrated diversion of most of the Fe59 given intravenously to the liver.2'4'8 The present data confirm these observations, even when the marrow is hyperplastic (patients IV and VI) and is reflected in the hemosiderosis of the liver observed at necropsy in patients I, II, IV, V, VI, and VII.

In patients II, III, and V there was also considerable diversion of Fe59 to the spleen. Patient I had only slight uptake of Fe59 by the spleen which weighed 200 Gm. at autopsy. The uptake of a large amount of Fe59 by an enlarged spleen is probably related to the observed hemosiderosis.

The plasma iron turnover in this series (table 1) was increased except in cases V and VII. Contrary to previous studies in other disease states the plasma iron turnover in these patients did not correspond to the rate of production of red cells, principally because the appearance of radioiron in circulating red cells was low. In agreement with the conclusions of Joske et al.15 the plasma iron turnover is not always useful in the evaluation of erythropoiesis. It has been shown that storage iron may be mobilized16 and has a turnover.17 The plasma iron turnover in the present series is a measure of iron transport within the reticuloendothelial system. The red blood cell iron turn-
over, however, more adequately measures erythropoiesis in our series except for case III. In this case the extremely high plasma iron turnover led to a calculated value for red cell iron turnover that appears to be high.

**Red Cell Life Span—Glycine C¹⁴ and Cr⁵¹ Studies**

The extent of incorporation of C¹⁴ from labeled glycine into hemoglobin agrees with the Fe⁵⁹ incorporation in those patients in whom a C¹⁴ glycine study was done (patients II, IV, V, VII), as has been previously demonstrated in acute leukemia.¹⁸ This indicates that the low uptake of radioiron in red cells reflects failure to synthesize hemoglobin and is not due to dilution in greatly expanded body iron stores.

All of the patients had decreased Cr⁵¹ red cell survival half-times, with evidence of splenic accumulation of Cr⁵¹ in patients II, III, and VI and of accumulation in the liver of patients I and VI. The direct and indirect Coombs’ tests were negative in all patients.¹⁸ There is also suggestive accumulation of Cr⁵¹ in the spleen of patient I and in the liver of patients III and IV. That the shortened survival was not due to blood loss was confirmed by frequent examinations for gross and occult blood in the stool and in patient VII by Cr⁵¹ counting in the stool. The only other visible sites of blood loss during the study were petecheiae and ecchymoses.

The interpretation of the Cr⁵¹ survival times in these patients is more complex than in patients with normal erythropoiesis. The Cr⁵¹ survival half-time values reported were obtained from the plot of Cr⁵¹ specific activity in whole blood. The graphs for Cr⁵¹ content per ml. red cells demonstrated (except in case I) rather constant specific activity until a transfusion was given. There was then an abrupt decrease in specific activity followed by resumption of an approximately constant but lower specific activity. The isotope content (Cr⁵¹/ml. RBC) decreases because of elution of Cr⁵¹ from intact red cells and replacement of effete or otherwise removed red cells by unlabeled red cells. In the absence of erythropoiesis, the isotope content per ml. red cells decreases only by elution from intact red cells and transfusion of unlabeled cells.

If, as a result of transfusions, the total red cell volume is maintained at an approximately constant level, then the unlabeled donor red cells usually supplied by the bone marrow do decrease the isotope content per ml. red cells and the time required to decrease by one-half the isotope content would be the equivalent of the survival half-time of Cr⁵¹ in red cells as ordinarily measured. The progressive decline in Cr⁵¹ content/ml. whole blood while the isotope content/ml. RBC stays approximately constant suggests that the plasma volume is increasing, tending to maintain the blood volume more constant than either the total red cell volume or plasma volume.

In patients II, IV, and VII, although the uptake of C¹⁴ into hemoglobin was low, an approximation of the red cell life span may be made. In a similar manner, either because of failure to reutilize erythrocyte iron or because of further decrease in the rate of erythropoiesis in four patients, the uptake of Fe⁵⁹ into peripheral red cells may be used to estimate the red cell life span. It must be

*In patient IV, only indirect Coombs’ test was done.
recognized that these are at best approximations, and that no method of measuring red cell life span will be entirely satisfactory in these clinical states.

Excluding patients III and VI, there was fair agreement between histologic evidence of erythropoiesis and measured red cell iron turnover. That is, those patients with cytologic evidence (either biopsy or autopsy) of erythropoiesis had a red cell iron turnover about one-third to one-half normal while those patients without anatomical evidence of erythropoiesis had much lower red cell iron turnover rates. Patient III with a very rapid plasma iron turnover and patient VI with an increased number of normoblasts in the bone marrow were unusual in this regard.

Effects of Therapy on Erythrokinetics

Androgen therapy for anemia, including aplastic anemias, has been reported with infrequent but occasionally spectacular benefit. The reported doses of androgen, usually testosterone propionate, were small in comparison to those suggested to us by Dr. Frank Gardner. Patient II with myeloid metaplasia, who received this therapy late in the course of his illness, showed no improvement. Patient V with myelosclerosis showed a slight but significant rise in her platelet count after androgen therapy was instituted.

The effect of androgen therapy in patient VII is equally difficult to evaluate. The changes seen might occur during the natural history of the disease. The results, however, are suggestive of a beneficial therapeutic effect.

Summary

1. Erythropoiesis was studied in seven patients with refractory anemia.
2. In all seven patients the total red cell volume was low and the plasma volume elevated.
3. The serum iron was elevated and iron binding protein saturated in six of seven patients.
4. The $T_{1/2}$ for clearance plasma Fe$^{59}$ was decreased in one, normal in two, and increased in four patients.
5. The red cell iron turnover was decreased in six of seven patients.
6. Radioiron accumulated in liver and spleen.
7. Red cell life span was difficult to measure but probably shortened.
8. Androgen therapy in two cases was ineffective, and was associated with a slight effect in one patient.

Summario in Interlingua

1. Le erythropoiese esseva studiate in septe patientes con anemia refractori.
2. In omne le septe, le total volumine erythrocytic esseva basse; le volumine del plasma esseva elevate.
3. Le ferro del sero esseva elevate, e le proteina ferro-ligante esseva saturate in sex del septe patientes.
4. Le periodo de medie valor pro le clearance ab le plasma de Fe$^{59}$ esseva reduce in un, normal in duo, e prolongate in quatro patientes.
5. Le metabolismo de ferro erythrocytic esseva reduce in sex de septe patientes.
6. Ferro radioactive se accumulava in le hepate e le splen.
7. Le longevitate del erythrocytos esseva difficile a mesurar, sed illo esseva probabilemente reducite.
8. Therapia a androgenos esseva inefficace in duo casos; illo produceva un leve effecto in un tertie.

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REFERENCES

Norman R. Gevirtz, M.D., National Heart Institute Postdoctoral Fellow, Department of Medicine, New York University School of Medicine, New York, N.Y.; formerly Clinical Associate, Metabolism Service, National Cancer Institute, Bethesda, Md.

Nathaniel I. Berlin, M.D., Ph.D., Clinical Director, National Cancer Institute, Bethesda, Md.
Erythrokinetic Studies in Severe Bone Marrow Failure of Diverse Etiology

NORMAN R. GEVIRTZ and NATHANIEL I. BERLIN