Erythrokinetics in the Megaloblastic Anemia of Tropical Sprue

By THOMAS W. SHEEHY, MILTON E. RUBINI, RAUL BACO-DAPENA AND ENRIQUE PÉREZ-SANTIAGO

ONE OF the primary manifestations of the clinical entity tropical sprue is a severe megaloblastic anemia. Although detailed descriptive information is available on the blood and bone marrow morphology of this anemia, its pathogenesis remains obscure. The purpose of this report is to describe the quantitative aspects of red cell production and destruction in this anemia. Our observations indicate that the immediate causes of the anemia of tropical sprue are (1) decreased delivery of erythrocytes to the peripheral circulation because of bone marrow dysfunction, and (2) increased destruction of intrinsically defective erythrocytes.

MATERIAL AND METHODS

General

Studies were performed on 10 selected Puerto Rican patients with untreated tropical sprue. All had classic manifestations of the disease, namely, severe macrocytic anemia, megaloblastic bone marrow, malabsorption with steatorrhea and diarrhea, and generalized malnutrition and weight loss. All subsequently had a hematologic remission following folic acid therapy. During hospitalization the patients were maintained on a preliminary "sprue diet" which is similar to the home diet of many patients with sprue. Control studies were done on 15 healthy Puerto Ricans recently inducted into the military service from civilian life.

Red blood cell counts, hemoglobin determinations and hematocrits were performed initially, and every 3 days thereafter, by the methods indicated. Bone marrow specimens were aspirated from the posterior iliac crest. The erythroid/myeloid ratio (EMR) of each marrow was obtained by counting 2000 cells. Only myelocytic and erythroid cells were counted, and the erythroid cells were used as an expression of erythropoiesis. Gross estimation of bone marrow hemosiderin was made on the basis of the Prussian blue reaction. A quantitative appraisal of the bone marrow iron content was made from 50 oil immersion fields. If no iron was present, the amount was classified as "0." The presence of intracellular iron granules or blue-staining reticulums in 1 to 5 fields was classified 1+, in 10 to 20 per cent of the field 2+ (normal), in 50 per cent or more of the fields 3+ and in all fields 4+. A spectacular degree of iron excess in all fields was classified 5+. Sideroblasts were stained and enumerated by the method of Kaplan. Serum iron was measured by the method of Ramsey, serum bilirubin by the method of Malloy and Evelyn. A direct antiglobin (Coombs') test employing serial dilutions was done on each patient. Three consecutive stools were examined at the beginning and end of each study for occult blood, ova and parasites.

Red Cell Survival and Ferrokinetic Studies

Sodium chromate (Cr⁵¹) with a specific activity of 50 μCi per milligram and ferrous citrate (Fe⁵⁹) with a specific activity of 5 to 15 μCi per milligram were administered to

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each patient, as well as whole blood or plasma analyzed for Cr\textsuperscript{51} and Fe\textsuperscript{59} radioactivity. Use of a single channel pulse height analyzer in conjunction with a well-type scintillation detector permitted simultaneous study with both isotopes in each patient.\textsuperscript{16} Counting efficiency was 2.9 per cent for Cr\textsuperscript{51} and 5.6 per cent for Fe\textsuperscript{59}. All samples were counted at the end of the collection period to avoid corrections for isotope decay. Erythrocyte survival of autogenous cells, and the red cell mass (RCM) were calculated with the use of a modification of the method of Donohue.\textsuperscript{17} The time required for one-half of the Cr\textsuperscript{51} activity to disappear from the circulation is expressed as $T_{1/2}$ Cr\textsuperscript{51}. This was corrected for elution in order to express the mean cell life (MCL) of the erythrocytes.\textsuperscript{17}

The plasma iron clearance rate was obtained by measuring the Fe\textsuperscript{59} activity in plasma samples drawn 10, 20, 30, 45, 60, 90, 120 and 180 minutes after the injection of Fe\textsuperscript{59}. The counts per minute measured for each plasma sample were plotted against time on semilogarithmic paper. The zero time plasma value was obtained by extrapolating the slow component of the plasma activity curve and using it as the 100 per cent value. The time required for one-half of the original radioactivity to disappear is expressed as $T_{1/2}$ Fe\textsuperscript{59}. Plasma iron turnover (PIT) is expressed as milligrams per 100 ml. whole blood per 24 hours, according to the method of Bothwell.\textsuperscript{18} This allows comparison of the PIT between two or more individuals of different sizes. The percentage of Fe\textsuperscript{59} incorporated into erythrocytes was measured with the use of a modification of the method of Giblett and is expressed as percentage of iron utilization (IU).\textsuperscript{19}

**Indexes of Erythrocyte Production and Destruction**

Total marrow erythroid activity was determined in each patient from the plasma iron turnover and the erythroid/myeloid (EMR) ratio.\textsuperscript{10,18} Effective erythropoiesis was estimated from percentage IU. Red cell destruction was estimated by observing the survival of Cr\textsuperscript{51}-labeled autogenous erythrocytes. The daily mass of erythrocytes destroyed each day was determined by dividing each patient's RCM by the MCL.

The erythrokinetic data on each patient were compared to normal by the use of arbitrary indexes described in detail elsewhere.\textsuperscript{10,18,19} The formulas used to determine the indexes of red blood cell production and destruction are listed below:

**Production**

\[ \text{total erythropoiesis} \]

1. Marrow erythroid/myeloid ratio index (EMRI) =
   \[
   \frac{\text{EMR p}}{\text{EMR n}} = \frac{465 \text{ erythroid cells/1000 myeloid cells}}{\text{EMR n}}
   \]

2. Plasma iron turnover index (PITI) =
   \[
   \frac{\text{PIT p}}{\text{PIT n}} = \frac{0.78 \text{ mg./100 ml. whole blood}}{\text{PIT n}}
   \]

**Effective erythropoiesis**

1. Red cell utilization Fe\textsuperscript{59} =
   \[
   \frac{\text{PIT p} \times \% \text{IU p at 14 day}}{\text{PIT n} \times \% \text{IU n at 14 day}} = \frac{PIT p \times \% \text{IU p at 14 day}}{0.78 \text{ mg./100 ml. whole blood} \times 93\% \text{ at 14 day}}
   \]

*These indexes were used to permit comparison with data in the literature on pernicious and other anemias.\textsuperscript{10} \textsuperscript{19}

*\textsuperscript{p} denotes the patient's value; \textsuperscript{n} denotes the value of the average control.
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DESTRUCTION

1. Marrow destruction index

\[ \text{EMR} \times \frac{\text{RBC}}{\text{EMRI} \times 5 \times 10^6 \text{ RBC/cu.mm.}} = \frac{\text{EMR}}{\text{RBC}} \times \frac{\text{EMRI} \times 5 \times 10^6 \text{ RBC/cu.mm.}}{\text{RBC}} \]

hemolytic index

1. Rate

\[ \frac{\text{MCL} \times 120 \text{ days}}{\text{MCL} \times \text{MCL}} = \frac{\text{MCL} \times 120 \text{ days}}{\text{MCL} \times \text{MCL}} \]

2. Mass

\[ \frac{\text{RCM} \times \text{MCL} \times 120 \text{ days}}{\text{MCL} \times \text{RCM} \times 30 \text{ ml. Kg.}} = \frac{\text{RCM} \times \text{MCL} \times 120 \text{ days}}{\text{MCL} \times \text{RCM} \times 30 \text{ ml. Kg.}} \]

RESULTS

The initial blood values of each patient at the time isotope studies were undertaken are listed in table 1. All but one patient had a severe anemia (hemoglobin less than 7.5 Gm.). Their serum iron and serum bilirubin values were normal.

Although the serum iron was not increased, storage iron was increased. As in pernicious anemia, the marrow iron was usually granular, although the amorphous variety was present. Granules were present singly and in aggregates of 15 or more. Intracellular iron was found in 40 to 60 per cent of the hemoglobin containing erythroblasts, the so-called “sideroblasts.” There was no increase in peripheral siderocytes, the average being 0.3 per cent, and none of the patients had significant reticulocytosis prior to therapy.

The results of the radioisotope studies are listed in table 2. The RCM was reduced in all patients, and the estimated plasma volume usually increased. Erythrocyte survival was abnormal in 8 of the 10 patients (fig. 1). Plasma iron clearance varied, and half the patients had a slight acceleration in their rate of Fe\(^{59}\) plasma clearance. Iron turnover (PIT) was increased in 7 of the 10 patients. Red blood cell incorporation of Fe\(^{59}\) was variable (fig. 2). Five patients had poor iron utilization, incorporating between 4 and 22 per cent of

Table 1.—Hematologic Measurements in the Anemia of Tropical Sprue

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age, race, sex</th>
<th>RBC (millions/cu.mm.)</th>
<th>Hgb. (Gm.%)</th>
<th>Hct. (%)</th>
<th>MCV (cu. µ)</th>
<th>Reticulocytes (%)</th>
<th>Bone marrow iron (µg.%)</th>
<th>Serum iron (µg.%)</th>
<th>Serum bilirubin (µg.%)</th>
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<td>3+</td>
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<td>15.0</td>
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<td>14.0</td>
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<td>0.7</td>
<td>4+</td>
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Table 2.—Erythrokinetic Data on Patients with Megaloblastic Anemia of Tropical Sprue

<table>
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<tr>
<th>Case no.</th>
<th>Red cell volume (mL/Kg.)</th>
<th>Plasma volume (mL/Kg.)</th>
<th>$T_{1/2}$ Cr$^{51}$ survival (days)</th>
<th>Plasma iron clearance ($T_{1/2}$ Fe$^{59}$) (min.)</th>
<th>Fe$^{59}$ turnover (mg/100 ml. W. B./day)</th>
<th>Fe$^{59}$ incorporation (% in 14 days)</th>
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<td>20.0</td>
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<td>15.0</td>
<td>34</td>
<td>1.17</td>
<td>88</td>
</tr>
</tbody>
</table>

Control

Mean: 30.2 37.2 27.3 ± 1.2 82.6 0.78 93
Range: 24.9–35.7 33.7–46.1 24.9–36.2 47–128 0.53–0.93 83–96

Fig. 1.—Erythrocyte survival in patients with the megaloblastic anemia of tropical sprue.

the tracer after 14 days. Two patients had curves suggesting decreased red cell production. They required 6 to 8 days to incorporate 50 per cent of the Fe$^{59}$ into their circulating erythrocytes, whereas the controls required only 3.5 to 4 days to incorporate a similar amount.

Finch has suggested that the bone marrow EMR and the PIT primarily reflect total marrow erythropoiesis and hemoglobin production, whereas erythrocyte utilization of Fe$^{59}$ reflects effective erythroid marrow production or actual delivery of red blood cells to the circulating blood. In our patients with tropical sprue, total erythropoiesis was increased, ranging from 2 to 4 times...
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Fig. 2.—Erythrocyte Fe\textsuperscript{59} uptake in patients with megaloblastic anemia of tropical sprue.

normal when estimated from the EMRI. It was increased in 8, normal in one and decreased in one patient when estimated by the PIT.* However, the actual delivery of red blood cells—or effective erythropoiesis—was usually reduced (table 3).

Red cell destruction as judged from $T_{1/2}$ Cr\textsuperscript{51} was increased to six times normal in one patient, three times normal in three, two times normal in four, and was normal in two. Because of the low volume of erythrocytes available for destruction, the mass of cells destroyed each day was usually not excessive (0.15 to 0.50 ml./Kg.) and was actually reduced in 4 patients (table 3).

**DISCUSSION**

The predominant defect in the megaloblastic anemia of tropical sprue is failure of effective erythropoiesis. As in pernicious anemia, the attempt by the bone marrow to deliver erythrocytes is largely ineffective.\textsuperscript{21}

\*The PIT may not reflect total marrow activity. The amount of iron available to the erythroblasts, to judge from the appearance of the bone marrow smears, was excessive, and the erythroblasts accepted excessive quantities of iron, more than they were capable of using for hemoglobin synthesis. This was evident by the granules of residual iron in the many sideroblasts observed in the marrow. When these erythrocytes are released to the circulation, the spleen removes the iron granules,\textsuperscript{22} and this iron is probably returned to the marrow via the plasma. The effect of this recycling of siderocyte iron (rather than increased total marrow activity) may account for the increased PIT observed in these patients.
### Table 3. Erythrogenic Indexes in the Megaloblastic Anemia of Tropical Sprue

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Cells/1000 myeloid cells</th>
<th>Index</th>
<th>(mg./100 ml. WB)</th>
<th>Index</th>
<th>(% in 14 days)</th>
<th>Index</th>
<th>Cells/1000 myeloid corrected for RBC</th>
<th>Index</th>
<th>MCL (days)</th>
<th>Index</th>
<th>(1)</th>
<th>Red cell mass ml./kg. destroyed/day</th>
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Control values: 465, 1.0, 0.78, 1.0, 93, 1.0, 465, 1.0, 120, 1.0, 0.25, 1.0
In the anemia of sprue, the serum iron is usually not elevated, but storage iron is increased. Such a rise in marrow hemosiderin is common to many anemias, and is due largely to a shift of iron from the blood cells to storage. In sprue, a lack of two-thirds of the circulating hemoglobin is common, and such a deficiency releases approximately 1.5 Gm. of iron to the storage sites. Following therapy with folic acid, the marrow iron deposits decrease and may eventually become depleted. Two of our patients (cases 3 and 5) showed signs of iron deficiency following an initial response to folic acid. Supplemental iron therapy produced a secondary reticulocytosis and a rise in their serum iron levels to normal.

The serum iron represents a balance between iron derived from hemoglobin destruction and iron absorbed from the intestine. In sprue, the daily hemoglobin destruction does not reach the proportions seen in many hemolytic anemias, and daily hemoglobin destruction may be less than normal. Intestinal absorption of iron may be impaired, and parasitosis, which was present to a mild degree in all our patients, may further contribute to intestinal iron loss. Any increase in transport iron was not detected as an increase in serum concentration.

The bone marrow activity in sprue was evaluated by a variety of tests. The PIT and EMR gave results indicating increased marrow erythroid activity, but this activity was not reflected in the reticulocyte count, the utilization of iron or in the cell survival studies. Half of the patients had flat Fe\textsuperscript{57} utilization curves suggesting severe marrow dysfunction. Such curves are also found in hemochromatosis, but in sprue, iron stores are not sufficiently increased to explain such depressed utilization. Furthermore, therapy with folic acid promptly corrects erythrocyte iron utilization. This is illustrated in figure 3. Prior to therapy this patient (case 3) required 20 days to incorporate 22 per cent of a tracer dose of Fe\textsuperscript{57}. The administration of only 25 μg. of folic acid resulted in a tremendous outburst of erythropoiesis, and rapid incorporation of 100 per cent of a fresh tracer dose of radioiron. As effective delivery of red cells from the marrow was enhanced, the reticulocyte count rose to 12 per cent, and as iron was utilized more efficiently, the plasma iron turnover decreased.

Giblett has noted that the relationship between the production and destruction of erythrocytes is not as clear-cut in patients with marrow dyspoiesis as in certain hemolytic anemias. This relation may be explained by the following observation. One patient (case 8) had volunteered for prolonged observation and was hospitalized initially because of diarrhea and gastrointestinal complaints. Absorption studies and a biopsy of the small intestine were consistent with a diagnosis of sprue. Although a megaloblastic bone marrow was found on admission, the patient did not have a severe anemia, and during the first few weeks of observation his hemoglobin was stable at 11.0 Gm./100 ml. Initial studies revealed a RCM of 22.4 ml./Kg., a T\textsubscript{1/2} Cr\textsuperscript{51} of 27.2 days and an IU of 66 per cent after 26 days. The slope of the IU curve suggested decreased erythrocyte production, and after the first month of observation the patient’s hemoglobin level began to fall, reaching 4.4 Gm. after 102 days. During this time there was no reticulocytosis or hyperbilirubinemia,
and no abnormal serum agglutinins or hemolysins were demonstrated. After 3½ months the RCM was 7.4 ml./K.g., the T<sub>1/2</sub> Cr<sup>51</sup> was 16.8 days and iron utilization negligible, only 3 per cent of the Fe<sup>59</sup> appearing in the peripheral erythrocytes after 10 days (fig. 4). Effective delivery of erythrocytes had decreased markedly, and the red blood cells delivered were living a shorter life span. At this time a marked megaloblastosis was evident on marrow examination. Although effective erythroid production had decreased, bone marrow production had not halted abruptly nor completely as in an aregenerative crisis, and red cells, platelets and leukocytes were being delivered to the peripheral blood but in decreasing numbers. Erythrocyte survival had decreased, but the actual number destroyed daily had changed little, falling from 23 to 19 ml. of erythrocytes per kilogram of body weight daily. Therefore, the amount of hemoglobin destroyed daily was not increased, and neither the serum bilirubin nor the serum iron levels rose.

The decreased survival of this patient’s erythrocytes was attributed to the production of defective cells. An intrinsic rather than extrinsic corpuscular defect was implied by the prolonged period required for the anemia to develop, the absence of serum agglutinins or hemolysins and the subsequent normal survival of fresh donor erythrocytes. Splenic sequestration could explain the random destruction of cells indicated by the exponential survival curves observed, and this is currently under investigation. It is reasonable to assume that as marrow dysfunction or arrest ensues in sprue, an increasing proportion of the cells produced are defective. However, the absence of an early fall in the iron incorporation curves indicates the erythrocytes are not destroyed immediately after their release from the marrow.

In an autopsy review of tropical sprue, Koppisch described several types of
bone marrow, ranging from diffuse megaloblastic hyperplasia to marked hypoplasia. We have never seen the latter type of bone marrow even in the severest case of sprue, but feel that case 5 was approaching complete functional disruption of marrow activity. On admission, he had severe pancytopenia, marked prolongation of plasma iron clearance (T1/2 Fe59, 498 min.), and marked reduction in plasma iron turnover. Effective erythropoiesis was decreased markedly but erythroid hyperplasia was still evident on bone marrow examination. An aregenerative or delivery crisis had developed, and delivery of all marrow elements decreased. Folic acid therapy promptly terminated the crisis and induced effective erythropoiesis.

The composite data indicate that in the megaloblastic anemia of tropical sprue a vicious cycle ensues as the anemia develops. The bone marrow attempts to maintain and increase hemoglobin production, but its efforts never approach the maximum activity observed in certain hereditary anemias. As the anemia progresses, effective erythropoiesis or the delivery of erythrocytes from the marrow decreases, and fewer and fewer cells reach the periphery. Moreover, an increasing proportion of those cells reaching the periphery
have a shortened life span. This further stresses the faltering marrow, and the anemia becomes more severe. In some cases an aregenerative crisis develops which completely eliminates effective production of all the marrow elements.

**Summary**

Blood production and destruction were measured in 10 patients with the megaloblastic anemia of tropical sprue. Methods employed included the determination of the erythroid/myeloid ratio of the marrow, plasma iron turnover, red cell utilization of Fe3+ and Cr51 red blood cell survival. Rates of production and destruction were compared to normal.

Patients with the megaloblastic anemia of sprue were usually not iron deficient. Total bone marrow erythroid activity did not approach the maximal response seen in other hemolytic anemias, and there was a marked decrease in the delivery of erythrocytes to the peripheral blood. The rate of red blood cell destruction was increased, but as the red cell volume decreased, the total mass of erythrocytes destroyed per day varied from less than normal to twice normal. Bilirubinemia was not marked, because the amount of hemoglobin destroyed daily was usually not excessive and excretory function was not impaired. The severity of the anemia was largely related to the erythrocyte production defect.

**Summario in Interlingua**

Le production e destruction de sanguine esseva mesurate in 10 patientes con le anemia megaloblastic de sprue tropic. Le methodos de investigation esseva le determination del proportion erythroide/myeboide in le medulla, le transition de ferro in le plasma, le utilisation de Fe3+ in le erythrocytos, e le longevitate erythrocytic secundo le metodo a Cr51. Le rhythmio del production e del destruction esseva comparete con le valores normal.

Le patientes con le anemia megaloblastic de sprue tropic habeva normalmente nulle carentia de ferro. Le total activitate erythroide del medulla non approchava le responsa maximal vidite in altere anemias hemolytic. Esseva notate un reduction marcate in le provision de erythrocytos al sanguine peripheric. Le rhythmio del destruction erythrocytic esseva accelerate, sed in tanto que le volumine del erythrocytos esseva reduceite, le massa total del erythrocytos destruite in le curso de un die variava ab infra normal usque a duo vices le valor normal. Bilirubinemia esseva pauco marcate, proque le quantitate de hemoglobina destruite in le curso de un die esseva usualmente non excessive, e la function excretori esseva intacte. Le severitate del anemia esseva relationate in grande mesura al defecto del production de erythrocytos.

**REFERENCES**

ERYTHROKINETICS IN ANEMIA OF TROPICAL SPRUE

Erythrokinetics in the Megaloblastic Anemia of Tropical Sprue

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