The Anemia of the Di Guglielmo Syndrome

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With the technical assistance of Angela Pasquarriello

A condition characterized by a generalized malignant proliferation of the nucleated red blood cells resembling leukemia was first recognized as a clinical and pathologic entity by Di Guglielmo in 1923; he termed it “acute erythremic myelosis.” In 1917, this author had described a mixed erythroblastic-leukocytic proliferation which he called “erythroleukemia.” However, in 1923, Di Guglielmo defined “erythremic myelosis” as “an autonomous pathologic entity, i.e., a primary and specific disease, characterized by a generalized proliferation of the erythropoietic cells of the bone marrow, analogous to the leukocytic proliferation in leukemia.” As the years went on, it became increasingly apparent that the “pure” erythroblastic proliferation described by Di Guglielmo in 1923 was rare, and that mixed forms, in which dual proliferations of both erythroblastic and myeloblastic elements were present, i.e., “erythroleukemia,” were much more commonly seen. This became more fully apparent when cases of apparently “pure” erythroblastic disorder were maintained for lengthy intervals with blood transfusions and antibiotics. Under these circumstances, the pure erythremic disorder often gave way to a mixed form, i.e., “erythroleukemia,” and these conditions often overlapped and merged. The longer the patient remained alive, the more likely was a final stage of pure acute myeloblastic leukemia to develop. Since the erythroblastic proliferation might appear before, during or even after the establishment of the leukemic picture in the peripheral blood, the impression increased that it probably represented only a single phase in the ultimate development of typical acute leukemia. Thus, one could think of “erythremic myelosis” as merely one aspect of a more generalized myeloproliferative disorder in which one or another cell of the bone marrow might participate either at various times, or simultaneously. Transient blood pictures and “mixed” forms could thus be readily understood on the basis of a total myeloproliferative disorder with different expressions in different individuals; and with variable expressions in the same individual.

Such thinking has tended to shift the emphasis from the large number of nucleated red cells in the blood to consideration of the bone marrow disorder as a whole. The occurrence of “anerythroblastic” forms, in which relatively...
few nucleated red cells are present in the peripheral blood despite the marked
erthroblastic hyperplasia of the marrow, may be likened to the now well-
known “aleukemic” form of leukemia. We have found, furthermore, that some
indication of myeloblastic proliferation is always present in the bone marrow
and often in the peripheral blood as well, either during the entire course of
the disease or in its later phases; even in the “pure” erythroblastic forms a
few myeloblasts accompanying the nucleated red cells in the peripheral blood
are usually seen. In most cases, the myeloblasts are quite numerous, thus
making sharp separation of “erythremic myelosis” from “erythroleukemia” impossible. Since these differently named conditions probably represent only
transitory clinical stages in the natural history of the same pathologic entity,
we have preferred to group all the various clinical variants into one entity
which we have termed the “Di Guglielmo syndrome.” The common dé-
nominators of the various entities or phases of this syndrome are a varying
degree of progressive anemia and an intense erythroblastic hyperplasia of the
marrow with the presence of “megaloblastoid” erythroblasts. Most of our cases
have passed through the following stages: (1) “pure” (or rather predomi-
nant) red cell proliferation; (2) mixed erythroblastic and myeloblastic pro-
liferation; (3) final “pure” or predominant myeloblastic proliferation, i.e.,
acute granulocytic leukemia.

In view of the striking erythroblastic proliferation of the bone marrow, the
anemia of the Di Guglielmo syndrome has aroused discussion, and the possi-
bility of a hemolytic component has been broached. However, the indications of outspoken hemolysis are usually lacking and the reticulocytosis is
only slight to moderate. Elevated fecal urobilinogen excretion values have
been reported in only a few cases; in these, an unusual degree of reticulo-
cytosis was present. Urobilinogen studies have not been recorded in the more
usual cases with minimal or moderate reticulocytosis; in these the possibility of
some type of “bone marrow failure” has usually been suspected.

Di Guglielmo speculated that the anemia was due to a combination of two
factors, i.e., the production of excessively fragile erythrocytes by the abnor-
ma]
erythroblastemia of varying degrees, and decreased, normal or slightly increased leukocyto-
sis with the presence of occasional to frequent myeloblasts.

Bone Marrow: Marked erythroid hyperplasia, anaplasia and dysplasia with (1) occa-
sional to many transitional forms, intermediate between reticulum cells and primitive
erythroblasts; (2) apparent erythroblastic maturation arrest at a primitive level; (3)
moderate to marked megaloblastoid features, asynchronism of nucleocytoplasmic matura-
tion, fine "scroll-work" like chromatin pattern of the nuclei; (4) increase in number of
mitoses with frequent abnormal mitotic figures; (5) degenerative features: homogeniza-
tion, segmentation and fragmentation of the nuclei; (6) a varying degree of myeloblastic
proliferation.

Effect of Therapy: Therapeutic trials with vitamin B₁₂ and folic acid were always with-
out effect on reticulocytes or red cell levels.

Hemolytic Studies

A group of tests to detect the presence and degree of erythrocyte destruction were
employed. These included:

1. Osmotic fragility by the method used in our laboratory.²⁶ By this method the incre-
ments of hemolysis at the various salt concentrations are plotted, and the presence of
variations in thickness of heterogeneous red cell populations are expressed graphically.

2. Reticulocyte count by the method of Dameshek.²⁵ Reticulocytes were expressed as
per cent of the RBC and as total number per cu. mm. of whole blood. The mean normal
values for this laboratory are 1.2 per cent or 60,000 per cu. mm.

3. Coombs' antiglobulin test, direct and indirect, and investigations of the sera for
iso- and autoagglutinins using bovine albumin and enzyme-treated red cells.²⁶

4. Filter paper electrophoresis of hemoglobin by the method of Smith and Conley.²⁹

5. Alkali denaturation test for hemoglobin by the method of Singer et al.²³

6. Serum iron determinations by the method of Ramsay.²⁷ Normal values for this labora-
tory are 110 to 165 µg./100 ml.

7. Serum bilirubin concentration by the method of Malloy and Evelyn.²⁹

8. Fecal urobilinogen excretion on pooled four-day stool collections by the method of
Watson.²⁹ The results were expressed in mg. urobilinogen excreted per day. From
these values hemolytic indices were calculated by the method of Miller et al.²⁶ The values for
hemoglobin mass required in the calculation of the hemolytic index were derived from the
red cell volume determined by the Cr²⁶ red cell-labeling method just prior to the deter-
mination of the Cr²⁶ red cell survival.

9. Survival of Cr²⁶-labeled red cells by the method of Read et al.²⁷ Twenty to 30 ml.
of patient's blood were added to 10 ml. of special AC₅ solution in a 100 ml. sterile
siliconized bottle* and incubated for 30 minutes with 75 to 85 microcuries of Cr²⁶ (as
Na₂CrO₄) at room temperature. The specific activity of Cr²⁶ averaged 52.8 mc./mg. The
chromated whole blood was infused into the patient. The first sample was taken 24
hours after the injection, and 6 to 10 samples were taken at various intervals until
the radioactivity in the circulating blood had decreased to less than 40 per cent of the
initial value. In six patients, in which the hemoglobin mass was also determined, the first
sample was taken 10 to 20 minutes after the injection of the labeled red cells. In two
other patients the first sample was taken 6 and 8 hours after the injection, respectivly.
Three-ml. blood samples were counted in a well-type scintillation counter.² Count ob-
tained were 20 to 40 times background and were reproducible within 2.5 per cent of
variation. Results were plotted against time on semilogarithmic paper, interpolated to
time zero (T₀) and expressed as percentage of counts at T₀. The normal red cell apparent
half-life (T₁/₂Cr²⁶) by this method, obtained in 10 medical students and physicians in
our laboratory, was 28.1 ± 2.3 days. From the T₁/₂Cr²⁶ values, the "mean red cell life"
(MCL) was calculated as described by Donohue et al.²⁸ with allowance for elution.
When the T₁/₂Cr²⁶ value was above 20 days the correction formula suggested by Hughes
Jones and Mollison²⁴ was used.

|Nancy Wood, Chicago.
The life span of the patients' red cells was determined in all patients. In five cases, the patients' labeled red cells were also injected into normal volunteers.

In several cases, production and destruction indices were employed to express the rate of blood production and destruction, respectively, in comparison to normal. These indices were obtained by the methods described by Finch et al., and the normal standards used in the calculations were those given by these authors. They corresponded very closely to the normal figures obtained in this laboratory. The calculation of production was accomplished by referring the patient’s data to his expected normal hematocrit, while destruction was referred to the actual hematocrit. The specific formulae employed were as follows:

**Production rate**

\[
\text{Marrow erythroid cells/1000 myeloid cells} \times \frac{120}{\text{Pt. hemoglobin (Gm.%)}} = \frac{\text{Pt. red cell survival}}{\text{Normal hemoglobin (15 Gm.%)}}
\]

**Destruction rate**

\[
\frac{120}{\text{Pt. red cell survival}} \times \frac{\text{Urobilinogen (mg./d./Gm. Hgb)}}{\text{Pt. actual circulating Hgb (Gm.)}} = \frac{\text{Pt. reticulocytes/100 RBC}}{\text{Normal hemoglobin (15 Gm.%)}}
\]

By dividing the patient's value of production or destruction for any one measurement by the corresponding normal value, production and destruction indices were obtained.

**Analysis of Results and Discussion**

Eleven patients, 26 to 70 years of age, were investigated in this study. Seven were considered to be acute and four chronic. Although the acute form of the Di Guglielmo syndrome is stated to be much more common than the chronic type, this may be due either to a lack of recognition of some of the chronic cases or to an overemphasis or overdescription of the relatively few acute cases that have been observed. In any event, the common denominator in both was marked anemia with a leukemia-like proliferation of erythroblasts in the bone marrow and other organs and a variable degree of release of these cells into the peripheral blood. The relationship between the stage of maturation of the nucleated red cells in the bone marrow and the clinical course of the patient was less well defined than in leukemia. Thus the distinction between the acute and chronic cases, if indeed any valid distinction existed, was usually made on the basis of the clinical symptoms and duration of the disease rather than on the hematologic findings.

In the acute cases, the course was rather brief (6 months to 1 year) with rapidly increasing anemia, in some instances with fever and hemorrhagic manifestations. The course of the chronic cases was often quite indolent; in fact, in one case (Case 6) documentation of the anemia—which was progressive—was available for a 10-year period from U.S. Army Medical Service records. No basic qualitative or quantitative differences between the acute and chronic cases were noted. In those cases in which the leukocytic portion of the bone marrow was greatly involved, the stage of immaturity of the

*Only myeloid and erythroid cells were counted. Lymphocytes, plasma cells, and reticulum cells were disregarded.*
TABLE 1.—Type and Degree of Anemia*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Patient</th>
<th>Clinical Course</th>
<th>R.H.C. (Millions per cu.mm.)</th>
<th>Hemoglobin (Gm./100 c.c.)</th>
<th>Hematocrit (%)</th>
<th>MCV (µL.)</th>
<th>MCH (µg.)</th>
<th>MCHC (%)</th>
<th>Red cell counts</th>
<th>Nucleated RBC (per cu.mm.)</th>
<th>Serum Văl. Bu. (mg./100 cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S.R.</td>
<td>Acute</td>
<td>2.33</td>
<td>6.8</td>
<td>26</td>
<td>111</td>
<td>29</td>
<td>26</td>
<td>7.0</td>
<td>2,050</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>L.O.</td>
<td>Acute</td>
<td>2.88</td>
<td>8.8</td>
<td>29.5</td>
<td>103</td>
<td>31</td>
<td>30</td>
<td>5.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>A.R.</td>
<td>Acute</td>
<td>2.11</td>
<td>7.4</td>
<td>24</td>
<td>114</td>
<td>35</td>
<td>31</td>
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<tr>
<td>4</td>
<td>R.F.D.</td>
<td>Acute</td>
<td>2.89</td>
<td>9.3</td>
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<td>104</td>
<td>32</td>
<td>31</td>
<td>10.0</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>G.M.</td>
<td>Acute</td>
<td>2.74</td>
<td>8.6</td>
<td>26</td>
<td>95</td>
<td>31</td>
<td>33</td>
<td>2.3</td>
<td>186</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>E.M.C.</td>
<td>Chronic</td>
<td>1.27</td>
<td>3.0</td>
<td>15</td>
<td>118</td>
<td>31</td>
<td>26</td>
<td>18.5</td>
<td>1,134</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>C.S.H.</td>
<td>Chronic</td>
<td>3.36</td>
<td>9.7</td>
<td>34</td>
<td>101</td>
<td>29</td>
<td>29</td>
<td>11.3</td>
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<tr>
<td>8</td>
<td>H.J.P.</td>
<td>Acute</td>
<td>2.54</td>
<td>8.1</td>
<td>29</td>
<td>114</td>
<td>32</td>
<td>28</td>
<td>3.4</td>
<td>48</td>
<td>1,100</td>
</tr>
<tr>
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<td>H.W.B.</td>
<td>Chronic</td>
<td>2.73</td>
<td>8.6</td>
<td>32</td>
<td>117</td>
<td>32</td>
<td>27</td>
<td>1.0</td>
<td>78</td>
<td>1,160</td>
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<tr>
<td>10</td>
<td>E.S.</td>
<td>Chronic</td>
<td>3.08</td>
<td>10.2</td>
<td>33</td>
<td>107</td>
<td>33</td>
<td>31</td>
<td>1.0</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>J.S.McD.</td>
<td>Acute</td>
<td>1.94</td>
<td>6.7</td>
<td>19</td>
<td>99</td>
<td>34</td>
<td>35</td>
<td>0.8</td>
<td>84</td>
<td>2,544</td>
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</tbody>
</table>

*Values encountered at time of study.

granulocytes and the number of myeloblasts present in the bone marrow were always more indicative of the acuteness of the process than was the character of the nucleated red cells.

In the present series, cases 1 and 11 were considered to be "pure" erythremic myelosis at the time of study; the other cases all showed a "mixed" erythroblastic-myeloblastic proliferation (erythroleukemia). Attempts to demonstrate different patterns of clinical and experimental disturbance depending upon differences in the type and physiology of the anemia as between the "pure" and "mixed" cases were unsuccessful.

Type and degree of anemia. Hemoglobin values in this series ranged from 3.9 to 10.2 Gm. per cent (table 1). Striking variations in size and shape of the red cells, together with various degrees of basophilic stippling and polychromatophilia were present; occasional target cells were usually seen. Calculation of the indices indicated that the anemia was "macrocytic" with a MCV increased from 95 to 118 cu. µ. Only in two instances was the MCV below 100 cu. µ.* Macrocytosis was frequently more marked in the more anemic patients and was possibly related to the megaloblastoid character of the erythroblasts in the bone marrow. However, in contrast with pernicious anemia, the MCH was not proportionately increased, being within normal limits in eight cases and only slightly elevated in three. Several of the patients had slight hypochromia as reflected by their low values of MCHC! (table 1).

No chemical abnormalities of hemoglobin were detected by the methods of paper electrophoresis or the alkali denaturation test. The starch block electrophoretic study performed in one case also gave normal result.

Bone marrow and peripheral blood smears from four patients were stained

*The normal MCV in our laboratory ranges from 82 to 96 cu.µ.
†The normal values of MCH and MCHC in our laboratory are 27 to 32 µg. and 32 to 36 per cent, respectively.
for the presence of mucopolysaccharides by the Hotchkiss method.\textsuperscript{36} Periodic acid-Schiff (PAS)-positive granules in the cytoplasm were found in variable numbers of erythroblasts in all four cases (fig. 1). However, the degree of positivity varied greatly from case to case; in one patient 32 per cent of the normoblasts in the bone marrow showed positive granules, while in another patient only 1.5 per cent showed this abnormal finding. The degree of positivity was not related to the degree of erythroblastic hyperplasia nor to the degree of megaloblastosis present. The percentage of PAS-positive erythroblasts was always higher in the bone marrow than in the peripheral blood preparations. The nature of the mucopolysaccharide substance giving the positive reaction in the Di Guglielmo syndrome has not been further investigated.

A positive PAS reaction of the erythroblasts was first described in Cooley’s anemia.\textsuperscript{37} In our control studies which included 5 normals and 31 other cases of different types of anemias, including P.A. and Cooley’s anemia, positive PAS granules were found only in Cooley’s anemia (4 of 6 cases).

All our cases showed a highly varying degree of erythroblastemia (from 48 to 7,300 per cu. mm.) both from patient to patient and in the same patient at various stages of the disease. No direct correlation could be established be-

\textsuperscript{*}The preparations were stained by Mr. John R. Baker, former head of the Cytochemistry Laboratory, Cancer Unit, New England Medical Center, to whom we wish to express our deep appreciation.
between the degree of erythroblastemia and the acuteness of the clinical course, nor was there any correlation between the degree of erythroblastemia and either the degree of anemia or the degree of erythrocytic destruction (tables 1 and 2). This observation indicates that the erythroblastemia found in the Di Guglielmo syndrome has no relationship either to the extent of hemolysis or to the response of the bone marrow to hemolysis. It is probable that the presence of nucleated red cells in the peripheral blood is due to the release of “unregulated” neoplastic cells by the abnormally proliferating bone marrow. Rohr has suggested that the erythroblasts in the peripheral blood of erythremic myelosis originate from neoplastic foci outside the marrow.27 A striking increase in nucleated red cells invariably followed splenectomy when this was done. The highest value for nucleated red cells in the blood was found in case 3, previously splenectomized.

The erythroid/myeloid ratio of the bone marrow was reversed in all the patients; in the acute cases the degree of erythroblastic proliferation was usually more intense. The number of erythroid cells per 1000 myeloid cells in one acute case was 16.7 times higher than normal (table 5). Always conspicuous, both in chronic and acute cases, was the striking increase in the numbers of primitive erythroblasts (erythrogones). In some cases, the great numbers of these forms with the relative paucity of mature erythroblasts suggested a condition of “maturation arrest.”

The greatest numbers of abnormal forms of erythroblasts were seen in the cases with the most intense erythrocytic proliferation. Typical megaloblasts or, if one wishes, “megaloblastoid” erythroblasts were seen in all the patients, although the degree of megaloblastic proliferation varied greatly from case to case.

The megaloblastic proliferation of the Di Guglielmo syndrome, in contrast with that of pernicious anemia, was completely resistant to liver, vitamin B12 and folic acid therapy. The serum vitamin B12 level was high. Thus, in three of our patients (Cases 8, 9 and 11), the serum vitamin B12 level was 1100, 1180 and 2544 µg per ml., respectively, distinctly above the normal range* (table 1). This test may conceivably be of value in some cases in which, at the time of examination, the differential diagnosis between the megaloblastosis of vitamin B12 deficiency and that of the neoplastic (Di Guglielmo) type offers some difficulties. The response to B12 therapy is of course a more practical although somewhat slower test. One may speculate that the abnormal megaloblastic proliferation of the nucleated red cells in the Di Guglielmo syndrome without response to B12 therapy is due either to an enzymatic fault in the metabolism of B12 or folic acid, or to an inability to utilize these factors. Thus, pernicious anemia in which there is a deficiency in vitamin B12 may be said to bear a certain relationship to the Di Guglielmo syndrome in which the megaloblastosis may be due to a defect associated with an inability to metabolize vitamin B12 or folic acid.

*By the Euglena gracilis method used here, the normal values are above 100 and below 1000 µg per ml. with a mean value of 120 to 150 µg per ml. The determinations were performed by Dr. Bernard A. Cooper of the Thorndike Memorial Laboratory to whom we express our deep appreciation.
Evidence of increased hemolysis. The Coombs' test was negative in all the cases, and investigation of the serum for iso- and autoagglutinins using bovine albumin and enzyme-treated red cells gave negative results. The indirect or delayed serum bilirubin was definitely above the normal level in only three patients. The excretion of fecal urobilinogen studied in six patients was found increased in all, with values for the hemolytic index ranging from 104 to 656 (normal, 11 to 22). The two patients showing the highest values of serum bilirubin (Nos. 6 and 8) had the highest values of fecal urobilinogen. It is of interest, however, that in other cases with definitely increased urobilinogen outputs the serum bilirubin values were normal (table 2). Splenomegaly of slight to moderate degree was present in 5 of the 11 cases. There appeared to be no relationship between the degree of splenomegaly, the duration of the disease or the extent of hemolysis (table 2).

The serum iron levels ranged between 140 to 375 µg. per cent. No direct relationship was present between this parameter and the red cell survival time (table 2).

Osmotic fragility curves revealed marked heterogeneity in the erythrocyte thickness population, possibly related to the striking variations in size and shape of the red cells as observed on inspection of the blood films (fig. 2). A good correlation could often be found between the increase in osmotic fragility and shortening of life span of the patients' red cells as measured with the Cr51-labeling technic (fig. 3).

The reticulocytes were increased in seven cases, normal in one and decreased in three cases. There was no relationship between reticulocyte values and the degree of anemia, although the highest reticulocyte values were found in patients with the greatest erythroblastemia. (tables 1 and 2). The degree of reticulocytosis did not reflect the rate of bone marrow red cell production, and wide discrepancies were noted between the “production index” as evaluated by reticulocyte counts and that indirectly derived from the red cell life span (table 3). It would seem that reticulocytosis in this disease is more the

### Table 2.—Evidence of Increased Hemolysis *

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Reticulocytes (1000's/mm.²)</th>
<th>Serum Bilirubin Indirect (mg.%</th>
<th>Fecal Urobilinogen</th>
<th>Cr¹ Survival</th>
<th>Serum Iron (µg.%)</th>
<th>Splenomegaly (cm. below LCM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>163</td>
<td>0.4</td>
<td>—</td>
<td>22</td>
<td>61</td>
<td>140</td>
</tr>
<tr>
<td>2</td>
<td>144</td>
<td>0.4</td>
<td>—</td>
<td>26</td>
<td>86</td>
<td>145</td>
</tr>
<tr>
<td>3</td>
<td>573</td>
<td>1.2</td>
<td>413</td>
<td>104</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>286</td>
<td>0.7</td>
<td>—</td>
<td>20</td>
<td>50</td>
<td>140</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>0.6</td>
<td>—</td>
<td>17</td>
<td>38.5</td>
<td>275</td>
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<td>235</td>
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<td>1,052</td>
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<td>19.1</td>
<td>47</td>
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<tr>
<td>7</td>
<td>410</td>
<td>1.0</td>
<td>—</td>
<td>15</td>
<td>32</td>
<td>220</td>
</tr>
<tr>
<td>8</td>
<td>86</td>
<td>1.4</td>
<td>740</td>
<td>236</td>
<td>8.3</td>
<td>14.5</td>
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<tr>
<td>9</td>
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<td>0.9</td>
<td>556</td>
<td>159</td>
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<td>50</td>
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<tr>
<td>10</td>
<td>33</td>
<td>0.7</td>
<td>459</td>
<td>106</td>
<td>—</td>
<td>80</td>
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<tr>
<td>11</td>
<td>16</td>
<td>0.8</td>
<td>242</td>
<td>98</td>
<td>27</td>
<td>96</td>
</tr>
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</table>

*Values encountered at time of study.
| MCL = Mean Cell Life of red blood cells.
| N¹⁵-glycine labelling method used.

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The increments of hemolysis of various salt concentrations were plotted. The curves obtained revealed marked heterogeneity in the erythrocyte thickness population.

consequence of aberrant erythropoiesis with release of immature red cells to the peripheral blood than a response to erythrocytic destruction.

The survival time of the patients' Cr$^{51}$-labeled red blood cells as measured in their own circulation was found to be shortened in 8 of the 11 patients, ranging from 8 to 22 days of T½. However, in only two cases was the T½ Cr$^{51}$ below 15 days (table 2). In three other patients it was within the normal range. If converted into values of mean cell life by appropriate formulae, these figures indicated a rate of destruction of 2.4 to 8.6 times normal (table 4). No direct relationship between the red cell life span and the degree of anemia could be noted (tables 1 and 2). In case 3, in which splenectomy was performed, no effect on red cell life span was observed as the result of operation.

The suggestion of Mollison$^{29}$ that the elution rate of Cr$^{51}$ from abnormal erythrocytes might be increased has not been supported by any experimental evidence. On the contrary, simultaneous studies using the Cr$^{51}$ and the Ashby technics have shown comparable results.$^{35}$ We have concluded that the Cr$^{51}$ method, if properly performed and evaluated, reflects the true destruction rate of the patient's own cells and is thus a valid method even in cases with highly abnormal red cells.

Causes of increased hemolysis. There can be no doubt that the major cause for the increased hemolysis of the Di Guglielmo syndrome is an "intrinsic" abnormality of the red cell. There is no indication of an immunologic mechanism, and the splenomegaly in 5 of the 11 cases was of only slight degree. Further-
Fig. 3.—A direct correlation could often be found between the increase in osmotic fragility and shortening of life span of the patient’s red cells as measured by the Cr51-labeling technic.

more, no correlation was found between spleen size and shortening of the erythrocyte survival time, and splenectomy in one case failed to produce a significant increase in the survival time of patient’s labeled erythrocytes.

The “intracorpuscular” red cell defect in our cases was reflected both in the anisocytosis of the erythrocytes and in the marked heterogeneity of the osmotic fragility curves (fig. 2). Although there appeared to be a correlation between the shortening of the erythrocyte life span and the increase in osmotic fragility of the erythrocytes (fig. 3), it cannot be stated that one was a consequence of the other.

A definite demonstration of the “intracorpuscular” red cell defect was made in five of our cases (Nos. 6-9, 11) by the transfusion of Cr51-labeled patient’s red cells into volunteers. In these experiments, the results obtained showed that even in a normal environment the patient’s red cells hemolyzed at an increased rate similar to that observed in the donor patients themselves.

“Heme diversion.” The fecal urobilinogen excretion and the life span of the red blood cells were simultaneously determined in six patients. It was found that the amount of fecal urobilinogen excreted could not be related to the degree of hemolysis as measured by the Cr51 technic. In only one case (Case 3) was the fecal urobilinogen output lower, but in the five other cases it was much higher than the amount which would have been expected from the degree of shortening of the red cell survival and the size of the hemoglobin mass (table 4). That the amount of fecal urobilinogen excreted was less than the amount “expected” from the shortening of the red cell life span is, in our experience,
Table 3.—Lack of Significance of Reticulocyte Values as a Measure of Red Cell Production

<table>
<thead>
<tr>
<th>Case No.</th>
<th>MCL* (Days)</th>
<th>Index of Production†</th>
<th>Absolute Index of 1000's per cu. mm.</th>
<th>Index of Production†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>0.9</td>
<td>163</td>
<td>3.2</td>
</tr>
<tr>
<td>2</td>
<td>86</td>
<td>0.82</td>
<td>144</td>
<td>2.9</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>4.2</td>
<td>573</td>
<td>13.3</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>1.5</td>
<td>286</td>
<td>6.2</td>
</tr>
<tr>
<td>5</td>
<td>38.5</td>
<td>1.8</td>
<td>62</td>
<td>1.3</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>0.67</td>
<td>235</td>
<td>4.8</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>2.4</td>
<td>410</td>
<td>7.3</td>
</tr>
<tr>
<td>8</td>
<td>14.5</td>
<td>4.5</td>
<td>86</td>
<td>1.8</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>1.37</td>
<td>27</td>
<td>0.6</td>
</tr>
<tr>
<td>10</td>
<td>80†</td>
<td>1</td>
<td>30.8</td>
<td>0.7</td>
</tr>
<tr>
<td>11</td>
<td>96</td>
<td>0.6</td>
<td>16</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*MCL = Mean Cell Life of red blood cells.
†Red blood cell production rate (Normal = 1).
‡N'5-glycine labelling method used.

Table 4.—Pyroline Pigment Metabolism

<table>
<thead>
<tr>
<th>Case No.</th>
<th>MCL* (Days)</th>
<th>Index of Destruction†</th>
<th>&quot;Hemolytic Index&quot;</th>
<th>Index of Destruction†</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>14</td>
<td>8.6</td>
<td>104</td>
<td>4.7</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>2.6</td>
<td>656</td>
<td>29.8</td>
</tr>
<tr>
<td>8</td>
<td>14.5</td>
<td>8.3</td>
<td>236</td>
<td>10.7</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>2.4</td>
<td>159</td>
<td>7.2</td>
</tr>
<tr>
<td>10</td>
<td>80†</td>
<td>1.5</td>
<td>106</td>
<td>4.8</td>
</tr>
<tr>
<td>11</td>
<td>96</td>
<td>1.2</td>
<td>98</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*MCL = Mean Cell Life of red blood cells.
†Red blood cell destruction rate (Normal = 1).
‡N'5-glycine labelling method used.

a common phenomenon in hemolytic anemias. However, in our cases, the opposite phenomenon was observed. Thus, in cases 10 and 11, the fecal urobilinogen output was 4.8 and 4.5 times higher than the maximum normal value, although no increased hemolysis could be detected. In patients 6, 8 and 9, the destruction rate of chromated red cells was 2.6, 8.3 and 2.4 times normal, whereas the urobilinogen hemolytic index was 29.8, 10.7 and 7.2 times the maximum normal value, respectively. A similar phenomenon is known to occur in pernicious anemia and is interpreted as "heme diversion," i.e., the excretion of pyrrole pigments not utilized for hemoglobin synthesis. Whether this phenomenon is due instead to rapid destruction of faulty red cells within the marrow and prior to their entrance into the peripheral blood remains unknown. Since no early fall in the red cell survival curves occurred in our cases, this seems unlikely. These observations of the lack of correlation between bile pigment output and the red cell life span lend further support for a functional similarity between the "deficiency" megaloblastosis of pernicious anemia and the "malignant" megaloblastosis of the Di Guglielmo syndrome.
ANEMIA OF THE DI GUGLIELMO SYNDROME

TABLE 5.—"Ineffective" Bone Marrow Erythropoiesis

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Nucleated RBC/1000</th>
<th>Index of Production*</th>
<th>MCL† (Days)</th>
<th>Index of Production*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>7,755</td>
<td>16.7</td>
<td>38.5</td>
<td>1.8</td>
</tr>
<tr>
<td>6</td>
<td>2,928</td>
<td>6.3</td>
<td>47</td>
<td>0.67</td>
</tr>
<tr>
<td>7</td>
<td>1,820</td>
<td>3.9</td>
<td>32</td>
<td>2.4</td>
</tr>
<tr>
<td>8</td>
<td>5,192</td>
<td>11.2</td>
<td>14.5</td>
<td>4.5</td>
</tr>
<tr>
<td>9</td>
<td>1,310</td>
<td>2.8</td>
<td>50</td>
<td>1.37</td>
</tr>
<tr>
<td>10</td>
<td>1,069</td>
<td>2.3</td>
<td>80†</td>
<td>1.1</td>
</tr>
<tr>
<td>11</td>
<td>1,470</td>
<td>3.2</td>
<td>96</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Red blood cell production rate (Normal = 1).
†MCL = Mean Cell Life of red blood cells.
‡N52-glycine labelling method used.

The depression of erythropoiesis. Whether the anemia of the Di Guglielmo syndrome is caused solely by hemolysis of imperfect erythrocytes or by bone marrow depression on the basis of defective maturation or delivery of cells was studied through the estimation of the erythrokinetic indices.25,26 The use of these methods for the evaluation of bone marrow erythrocytic production is valid only if the hematocrit is stable during the time of the experiment, i.e., if a steady state exists between production and destruction of red cells. This was the case in most of our patients; in others, although a slight variation was present during study, it is probable that it could not greatly affect the results.

The rate of red cell destruction as measured by the Cr51 method was in almost all cases insufficient to justify by itself the presence of anemia. In fact, in cases 2, 10 and 11 the red blood cell life span was within normal limits; in six others cases the rate of red cell destruction was 2 to 3 times normal, and in only two cases (3 and 8) were the values 8.6 and 8.3 times normal, respectively. It has been calculated that normal bone marrow production, as in typical hemolytic disorders, can compensate for a rate of red cell destruction 6 to 8 times normal.40 Of further interest was the fact that comparable rates of hemolysis were found with different degrees of anemia, and no inverse correlation existed between mean cell life of red cells and hemoglobin level in our patients. These various features pointed to the presence of a bone marrow defect, despite the increased proliferation of erythroid cells in the marrow. The production index calculated from the mean cell life of Cr51-labeled red cells was always lower, sometimes strikingly so, than that indicated by the erythroid/myeloid ratio of the bone marrow cells (table 5). The difference between these two indices expressed the range of the "ineffective" erythropoiesis in this disease, i.e., an increased proliferation of nucleated red cells in the bone marrow without a comparable increase of red cell production. "Ineffective" erythropoiesis reached highest values in Case 5, in which the greatest degree of erythroblastic hyperplasia was found.

Radioiron Studies. Further information regarding the presence and the degree of "ineffective" erythropoiesis was obtained in three additional patients more recently studied with the simultaneous use of Cr51 and Fe59.
The plasma iron turnover and red cell utilization were measured according to the method of Huff et al. Approximately 20 μc. of Fe⁺⁺ were injected intravenously, as ferrous citrate (approximately 7 μg of Fe), after incubation for half an hour with 30 to 40 ml. of fresh normal plasma. Numerous samples were taken soon after the injection and scattered samples were drawn every 2 to 3 days for the following 18 days. Twenty to 30 ml. of blood were labeled with Cr⁺⁺ by the method described above and injected into the patients soon after the Fe⁺⁺ clearance study had been completed. In the blood samples taken during the following days Cr⁺⁺ and Fe⁺⁺ were discriminated by the use of a pulse height analyzer. Ferrokinetic data and hemoglobin production indices were calculated according to Giblett et al. and Bothwell et al. The plasma Fe⁺⁺ clearance was found to be more rapid than normal. This was in contrast with the values for red cell utilization of Fe⁺⁺ which, expressed as per cent of the injected dose, were all lower than normal. Hemolysis, simultaneously measured with the Cr⁺⁺ method, was normal in one patient and moderately increased in the other two (table 6, fig. 4).

The hemoglobin production indices calculated from the plasma iron turnover and also those derived from the erythroid/myeloid ratio of the bone marrow were found to be considerably elevated. These values were consistently higher than the hemoglobin production indices calculated both from the red cell iron utilization and from the life span of the red cells, thus confirming the presence of "ineffective" erythropoiesis of marked degree.

These various data collected in different ways lend support to the concept that the erythroblastic hyperplasia in the Di Guglielmo syndrome may be the expression of an abnormally proliferating (neoplastic) tissue in which the anemia is due not only to increased hemolysis of faulty erythrocytes but to an ineffective type of erythropoiesis. In view of the megaloblastosis of the bone marrow, the high values for serum vitamin B₁₂, and the lack of response to both folic acid and vitamin B₁₂ therapy, one may speculate that a hitherto unrecognized metabolic defect of the erythron is present, perhaps due to an acquired inability to take up or to metabolize the B₁₂ vitamin. This is in line with the suggestion increasingly made that neoplastic proliferations may result from acquired enzymatic defects of diverse origin, eventuating in a highly abnormal type of growth pattern (Haddow). That this may occur on the part of the red cells, as well as of the white cells, seems increasingly likely, and in any event merits further study.

**SUMMARY**

The physiopathology of the anemia of the Di Guglielmo syndrome (crythremic myelosis) was studied in 11 patients with the acute and chronic varieties of the disease. Ferrokinetic studies were performed in three additional patients.

1. The anemia was normochromic and macrocytic; in contrast to the mean corpuscular volume, which was elevated, the mean corpuscular hemoglobin was often normal. In several patients the mean corpuscular hemoglobin concentration was slightly lower than normal, suggesting slight hypochromia.

2. Reticulocytes were often increased but bore no relationship to the degree of the anemia nor to the shortening of the red cell life span. The reticulocyte count is an unreliable index of blood production in this disease.

*RLI-4, Tracerlab, Waltham, Mass.*
Fig. 4.—The rapid course of the Fe²⁺ clearance contrasted with the low values for red cell utilization of Fe²⁺, thus confirming the presence of “ineffective” erythropoiesis of marked degree.

3. The degree of erythroblastemia was highly variable. No direct correlation existed between the degree of erythroblastemia and the acuteness of the disease, nor was there any relationship between the degree of erythroblastemia and either the degree of anemia or the degree of erythrocytic destruction.
TABLE 6.—Ferrokinetic Studies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hgb. Gm.%</th>
<th>Serum Iron μg./100 ml.</th>
<th>Plasma Iron Turnover T ½ Minutes</th>
<th>Turnover mg./100 ml. Blood/Day</th>
<th>% After 14 Days</th>
<th>T ½ Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. F.R.</td>
<td>10</td>
<td>160</td>
<td>53</td>
<td>2.14</td>
<td>40</td>
<td>29</td>
</tr>
<tr>
<td>2. A.K.</td>
<td>10.5</td>
<td>190</td>
<td>37</td>
<td>3.34</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>3. L.K.</td>
<td>8.9</td>
<td>120</td>
<td>42</td>
<td>2.20</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Normal Values</td>
<td>15</td>
<td>120</td>
<td>91</td>
<td>0.61</td>
<td>77</td>
<td>28.1</td>
</tr>
</tbody>
</table>

Hemoglobin Production Indices

<table>
<thead>
<tr>
<th>Patient</th>
<th>Bone Marrow</th>
<th>Plasma Iron Turnover</th>
<th>Red Cell Utilization</th>
<th>Cr² Size Surv.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. F.R.</td>
<td>3.1</td>
<td>3.5</td>
<td>1.8</td>
<td>0.7</td>
</tr>
<tr>
<td>2. A.K.</td>
<td>3.1</td>
<td>5.5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3. L.K.</td>
<td>2.4</td>
<td>3.6</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Total Erythropoiesis</td>
<td>Effective Erythropoieses</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. The bone marrow showed striking erythroblast hyperplasia. This was usually of the megaloblastic type. Primitive erythroblasts (erythrogones) were conspicuous. The erythroblast hyperplasia was out of proportion to the relatively minor reticulocytosis or the relatively slight diminution in red cell survival.

5. The nucleated red cells of the marrow showed variable numbers of megaloblasts and megaloblastoid forms, suggesting the presence of a vitamin B₁₂ deficiency (pernicious anemia). However, the vitamin B₁₂ concentration of the serum was elevated, and there was no response to the administration of vitamin B₁₂ or folic acid.

6. Varying numbers of erythroblasts in the bone marrow and in the peripheral blood showed periodic acid-Schiff (PAS)-positive granules in the cytoplasm. No chemical abnormalities of hemoglobin could be detected either by the method of paper electrophoresis or by the alkali denaturation test.

7. Diminished red cell survival was present in most cases, but it was of a relatively slight degree. It was due to an "intracorpuscular" defect of the red cells.

8. The often great increase in fecal urobilinogen output as compared with a relatively minor rate of red cell destruction suggests "heme pigment diversion" or increased destruction of precursor red cells, as in pernicious anemia, where the same phenomenon has been observed.

9. The great increase in the number of erythroid cells in the bone marrow and the increased rate of iron turnover as compared with the relatively minor increase in red cell destruction and iron utilization point to an "ineffective" type of erythropoiesis. The high degree of "ineffective erythropoiesis" seen in this disease may be characteristic of the neoplastic proliferation of the red cell series.

10. In conclusion, the anemia of the Di Guglielmo syndrome is due to a combined disturbance: (1) an "ineffective" type of erythropoiesis of marked degree, perhaps due to an acquired (neoplastic) defect in the uptake or utili-
ANEMIA OF THE DI GUGLIELMO SYNDROME

zation of B₁₂ by the erythroblasts and (2) increased hemolysis resulting from the increased destruction of defective red cells.

**SUMMARIO IN INTERLINGUA**

Le physiopathologia del anemia del syndrome de Di Guglielmo (myelosis erythremic) esseva studiate in 11 patientes con le varietates acute e chronic del morbo. Studios ferrocinetic esseva executate in tres patientes additional.

1. Le anemia esseva normochromic e macrocytic; per contrasto con le volume corpuscular medie, le qual esseva elevate, le hemoglobina corpuscular medie esseva frequentemente normal. In plure patientes le concentration medie del hemoglobina corpuscular esseva levemente plus basse que normal, lo que pote indicar un leve hypochromia.

2. Le numeration del reticulocytos esseva frequentemente elevate sed manifestava nulle relation con le grado del anemia o le reduction in le supervivintia del erythrocytos. In iste morbo le numeration de reticulocytos es un indice paucio fidel del production de sanguine.

3. Le grado de erythroblastemia esseva multo variabile. Nulle correlation directe existeva inter le grado de erythroblastemia e le acutessa del morbo, e etiam nulle relation existeva inter le grado de erythroblastemia e le grado de anemia o inter illo e le grado de destruction erythrocytic.

4. Le medulla ossee monstrava un frappante hyperplasia erythroblastic, que esseva generalmente del typo megaloblastic. Erythroblastos primitive (erythrogonos) esseva conspicue. Le hyperplasia erythroblastic esseva disproportionatemente plus pronunciate que le relativemente minor reticulocytosis, o que le relativamente leve reduction in le supervivintia del erythrocytos.

5. Le nucleate erythrocytos del medulla monstrava variabile numeros de megaloblastos e de formas megaloblastoide, lo que suggere le presentia de un deficientia de vitamina B₁₂ (anemia perniciose). Nonobstante, le concentration de vitamina B₁₂ in le sero esseva elevate, e il habeva nulle responsa al administration de vitamina B₁₂ o de acido folic.

6. Un variabile numero de erythroblastos in le medulla ossee e in le sanguine peripheric monstrava granulos positive a acido periodic Schiff in lor cytoplasm. Nulle anormalitates chimic del hemoglobina poteva esser detegite per le methodo de electrophorese a papiro o per le test del disnaturation alcalin.

7. Un reducite supervivintia del erythrocytos esseva constatate in le majoritate del casos, sed illo esseva de relativamente leve grados. Illo esseva debite a un defecto “intracorpuscular” del erythrocytos.

8. Le frequentemente grande elevation del production de urobilinogeno fecal, in comparation con le relativamente lente destruction del erythrocytos, suggere le occurrence of a “diversion del pigmento heme” o un augmentate destruction de erythrocytos precursori, como in anemia perniciose, ubi le mesme phenomeno ha essite observate.

9. Le grande augmento in le numero de cellulas erythroide in le medulla ossee, e le accelerate metabolismo de ferro in comparation con le relativamente minor augmento in le destruction de erythrocytos e in le utilisation de ferro
indica un typo "inefficace" de erythropoiese. Le alte grado de "erythropoiese inefficiente" vidite in iste morbo pote esser characteristic del proliferation neoplastic del serie erythrocytic.

10. In conclusion, le anemia del syndrome de Di Guglielmo es debite a un disturbation combine: (1) un typo "inefficace" de erythropoiese de grado marcate, possibilemente le resultate de un defecto acquirite (neoplastic) in le acceptation o utilisation de B₁₂ per le erythroblastos e (2) un augmentate hemolyse resultante del augmentate destruction de erythrocytos defective.

APPENDIX

Case 1: Susan R. (N.E.C.H. #99–714)

This 50-year-old married white woman was admitted October 10, 1955 with a history of anemia of 10 months’ duration. Various hematinsics (iron, vitamin B₁₂, folic acid, citrovorum factor) had been administered without effect. Since the basal metabolic rate was minus 20 per cent, thyroid had been given, but without effect on the blood picture. Shortly before admission a few petechiae and ecchymoses had been noted. Cortisone was then given without improvement.

The patient’s main complaint was increasing weakness and fatigue. In March 1955, the hemoglobin was 9 Gm., with the reticulocytes varying between 0.7 and 3 per cent. The WBC was 5,900 to 9,100, with the following differential count: mature polymorphonuclear neutrophils 49, band form polymorphonuclears 27, eosinophils 1, lymphocytes 19, monocytes 4 per cent. Platelets were adequate. The red cells differed markedly in size and shape, and the presence of basophilic and stippled red cells was noted. A palpable spleen was first detected in July 1955, and nucleated red blood cells in the peripheral blood were first found in September.

On admission to the New England Center Hospital, the patient showed marked pallor without jaundice. The spleen descended 2 cm. on deep inspiration, the liver was not palpable and there was no evident lymphadenopathy. Fading ecchymoses were noted on the lower extremities.

The blood counts showed: hemoglobin 6.8 Gm. per cent, RBC 2.3 M, hematocrit 26 per cent, WBC 11,300 with 54 polymorphonuclear neutrophils, 40 lymphocytes, 6 per cent monocytes. There were 35 nucleated red cells per 100 white cells. Platelets were 78,120 per cu. mm. Reticulocytes were 6.5 per cent. Marked basophilic granules and stippled red cells were noted. Poikilocytosis and anisocytosis were striking. Indirect serum bilirubin was 0.4 mg. per cent. A Cr² red cell survival study gave a T₁/₂ of 22 days. The Coombs’ test was negative. The sedimentation rate (Westergren) was 54 mm./1 hr. The serum iron was 140 µg. per cent. Hemorrhagic studies gave normal result; only the tourniquet test was slightly positive. Bone marrow examination showed a marked increase in cellularity with marked proliferation of the erythroid series. The M/E ratio was 1/5. Erythrogones were numerous, many very large in size and many with marked vacuolization of the cytoplasm. Binucleated forms were frequently found and mitoses were numerous. There was a relative decrease in number of the myeloid elements, but no increase in myeloblasts or other immature forms was noted. Megakaryocytes were decreased. A splenic aspiration revealed many erythrogones together with large and small nucleated red blood cells.

In summary, there was a brief history of progressive anemia with development of an enlarged spleen, presence of nucleated red blood cells in the peripheral blood with marked abnormalities in size and shape of the mature red cells, marked proliferation of erythroblasts in the bone marrow with many abnormal forms in the absence of acute signs of hemolysis and presence of many erythrogones and nucleated red cells in the spleen puncture material. This combination of findings suggested a proliferative disease of red cells involving the bone marrow and spleen. The patient was diagnosed as having acute erythremic myelosis of the "pure" type. During the hospital period, three blood transfusions were given with improvement of the hemoglobin level. On discharge, only
transfusion therapy was recommended. Unfortunately the follow up of this patient was not obtained.

Case 2: Leo O. (N.E.C.H. #100-891)

The patient, a 26-year-old white male, was first admitted to the New England Center Hospital on December 3, 1955, with the chief complaints of weight loss, weakness, fatigue and loss of appetite. The illness may have started four to five months before when the patient began to suffer from progressive easy fatigability and listlessness. Ten weeks before admission the patient had had a febrile episode with headache and cold for which he was treated with penicillin. He improved enough to return to work, but after four weeks the fever returned with cold, sore throat and cough. Penicillin again gave some improvement until five weeks before admission when the patient was hospitalized and found to have anemia. He was treated with four transfusions, but on his return home he noted general malaise, fatigue, chills and a high fever (to 104°). Three days prior to the hospital admission, a bone marrow puncture demonstrated acute leukemia.

Additional history revealed epilepsy of about 10 years' duration. The original seizures were fairly frequent (even 20 to 30 times a day), but at the age of 24 these attacks diminished and his last petit mal was two years before admission. He had been treated for this disturbance with anticonvulsants (Mebaral, Dilantin) for the past 10 years. The last treatment with Dilantin had been given in 1953. There was no history of head injury and no family history of epilepsy.

On physical examination, the patient appeared in good condition although his weight loss had been 30 pounds in the last year. Positive physical findings included moderate pallor and several scattered ecchymoses on the trunk and hips. No jaundice was noted. There was no lymphadenopathy or splenomegaly. The liver was palpable 2 cm. below the costal margin.

The laboratory data revealed the presence of pancytopenia with 2.8 M red blood cells, 300,000 platelets and 3,900 white blood cells. The reticulocytes numbered 5 per cent. The red cells on smear showed marked stippling and conspicuous variation in size and shape with, however, a normal complement of hemoglobin. Seven nucleated red cells per 100 white cells were counted. Some of these cells appeared abnormal, with large cytoplasm, resembling megaloblasts. Many white cells on the smear were immature; 20 myeloblasts and 5 promyelocytes per cent were counted. The Coombs' test was negative. Plasma hemoglobin was 16 mg. per cent. The bone marrow showed hypercellularity with increased numbers of myeloid and particularly of erythroid cells. The megakaryocytes were slightly decreased in number. The granulocytes showed a marked shift to the left with increased number of myeloblasts. The nucleated red blood cells were abnormal not only in number but also in morphology. These latter changes included (1) pleomorphism, (2) megaloblastoid appearance of nuclear chromatin, (3) dissociation of maturation of nucleus and cytoplasm, (4) pseudopodia of nucleus and cytoplasm, (5) increased number of erythrogonones, (6) increased numbers of mitotic figures. The diagnosis of acute erythroleukemia was made.

After unsuccessful therapy with vitamin B₁₂ and folic acid, which had no effect on the megaloblasts in the bone marrow, the patient was given large doses of Meticorten (1000 mg. daily). The dosage was progressively reduced after 22 days and finally was discontinued due to the patient's marked intolerance (agitation, depressed state) to this massive dosage of corticosteroids.

On January 13, 1956, the patient had an acute onset of fever, cough, chest pain, dyspnea and increasing weakness. A painful nodule appeared on the forearm and progressive ecchymoses and petechiae appeared all over the body. Five days later, in addition to the scattered ecchymoses and petechiae, a subcutaneous area of infiltration was seen in the right groin, and purpuric and necrotic areas were noted on the buttocks. The lips, mouth and gums showed scattered necrotic purpuric sores. Enlarged lymph nodes were noted in the left groin. The liver was enlarged, although it had not increased in size since the time of his last examination. The spleen was not palpable.

Blood studies revealed: hemoglobin 8.1 Gm. per cent, platelets 72,560, WBC 750
Numerous myeloblasts and nucleated red cells were found on the smears. Examination of the bone marrow revealed the erythroleukemic changes as previously described.

Treatment with achromycin and erythromycin for six days had no effect on the patient's fever. Six-mercaptopurine was administered for two weeks, but since no hematologic remission was obtained and increasing pancytopenia was noted the drug was discontinued.

The patient remained febrile during his entire hospital course and suffered discomfort from dyspnea, cough, thoracic and abdominal pain, intermittent diarrhea. X-ray examination of the chest indicated areas of infiltration in the left upper lobe and in both lower lobes. There was also right pleural effusion. Several bacteriologic studies gave negative results, and the infiltrations were thought to be due to leukemic infiltration or to infarctions. The patient died on February 2, 1956. Postmortem examination was not obtained.

Case 3: Alfred R. (N.E.C.H. #97-481)

This 52-year-old carpenter apparently had been in good health until a few months before admission when he felt progressive weakness and fatigue for which he was admitted to his local hospital. Anemia was diagnosed and the patient was treated with blood transfusions. Soon after discharge, weakness and fatigue reappeared associated with dizziness. Treatment with liver extract was given with no improvement. The patient had received a total of 11 blood transfusions in 9 months. On June 22, 1955, the patient was first admitted to the New England Center Hospital.

On physical examination, the patient was found in fairly good condition. Only pallor and splenomegaly (4 cm. below the costal margin) were found. No icterus and no petechiae were seen.

Repeated hemograms showed normochromic anemia (hemoglobin 8 Gm. per cent) and leukocytosis (20,000 WBC per cu. mm.) with a few immature granulocytes. Platelets were adequate. There was a striking reticulocytosis (25 per cent); anisocytosis, poikilocytosis, polychromatophilia were marked; macrocytes and a few micro- and spherical forms were seen on smear. The Coombs' test was negative. Serum bilirubin was slightly elevated (1.8 mg. per cent and 1.6 of the indirect variety). Serum iron was 375 gamma per cent and plasma hemoglobin was slightly increased (9.5 mg. per cent). There was a slight increase in hypotonic fragility of the red cells. The bone marrow showed a normal cellularity with increased erythroid hyperplasia. A few macronormoblasts were seen. No abnormalities of the white series were recorded.

On the basis of these data, it was felt that the patient might have hemolytic anemia of an ill-defined variety. Because of the increasing requirement of transfusions, splenectomy was performed. The spleen pathology showed changes consistent with hemolytic anemia and with myeloid metaplasia, the tissue being diffusely peppered with myeloid cells and particularly erythroblasts in all stages of development. The possibility of a leukemic infiltration was not ruled out. However, the liver tissue showed only minimal leukocytosis in the sinusoids.

The post-splenectomy blood picture showed a remarkable decrease of the reticulocytosis, and the hemolytic process seemed to have been favorably affected by the operation. However, a second bone marrow aspiration before discharge showed definite hyperplasia of red cell and white cell precursors and numerous mitotic figures. It was felt that the hemolytic anemia might have been secondary to an incipient myeloproliferative process.

After discharge the patient's hemoglobin was maintained at a higher level with the same amount of transfusions as given before. The blood picture, however, showed 50 to 85 nucleated RBC per 100 WBC, elevated reticulocyte count and essentially normal platelets, but there was a striking rise of the WBC which attained levels over 200,000. Bone marrow aspirations were repeated and a progressively increasing hyperplasia, particularly of the granulocytic series, was seen. The patient's physical condition was well maintained, however, and he was able to continue working.

In December, weakness increased and severe pain in the right upper abdominal quadrant and epigastric region appeared together with fever. There was extreme pallor and
slight icterus of the sclerae. Shotty small cervical and axillary nodes measuring 1 to 2 cm. in diameter were found. The liver was enlarged to the umbilicus.

Hemoglobin was 4 Gm. per cent and the WBC were 226,400 per cu. mm. with 15 per cent myeloblasts. There were 65 nucleated RBC per 100 WBC. Platelets were 160,000 per cu. mm. and reticulocytes were 18.8 per cent. A bone marrow aspiration showed definite immaturity and increased numbers of both granulocytes and erythroblasts. Many of the red cell precursors had megaloblastoid chromatoid structure, pseudopods and vacuoles. Increased mitoses were also noted. Reticulum cells were increased. Megakaryocytes were decreased.

It was concluded that the patient had a myeloproliferative syndrome involving both white and red cells, i.e., "erythroleukemia," acute in type.

While in the hospital, the patient was treated with 6-mercaptopurine (200 mg. daily) and showed a marked clinical and hematologic improvement on this regimen. In two weeks platelets rose to 670,000. The patient was also given four units of blood. The reticulocyte count went down to 4 per cent. Nucleated red cells and myeloblasts in the peripheral blood decreased in number.

Following discharge in January 1956, the patient was unable to return to work because of weakness and extreme fatigability. On 6-mercaptopurine the leukocyte count diminished to 10,000 per cu. mm. and platelets persisted at a normal level, but myeloblasts and nucleated RBC never disappeared completely from the peripheral blood. The transfusion requirement was of an average of three transfusions per month.

In July, the patient was seen again at the New England Center Hospital. In the weeks preceding this admission the WBC had risen again to 328,000 with 80 per cent myeloblasts and 140 nucleated RBC per 100 WBC. Therapy with 6-mercaptopurine was again effective, and at the time of discharge there were only 8 myeloblasts per cent with a WBC of 9600 per cu. mm. The decrease of nucleated RBC was proportional to that of the WBC and showed 140 per 100 WBC.

During this hospitalization the patient had an episode of acute pharyngitis with fever. The physical condition showed progressive deterioration with weight loss and congestive heart failure. At discharge, the patient's condition was little improved.

A few days after discharge, the patient developed furunculosis of the forehead which rapidly spread over the face and neck. Lymph nodes of the neck, axillae and groins enlarged rapidly and became painful. A persistent and very sharp pain in the right hip then developed and fever increased.

On readmission to the New England Center Hospital in August 1956, the patient appeared to be in a terminal state. Marked and painful generalized lymphadenopathy was found. The liver was below the umbilicus. There was bilateral ankle edema. Furunculosis was widespread over the face and neck and the patient was febrile.

Hemoglobin was 7 Gm. per cent, the WBC was 305,000 with great numbers of myeloblasts. Many of the nucleated red cells in the peripheral blood were very immature. Platelets were normal. Reticulocytes had dropped to 0.1 per cent. Stool guaiac test was found positive although there was no real evidence of gross hemorrhage. Petechiae were not found. The electrocardiogram showed great abnormalities consistent with pericardial infiltration. There was marked edema. X-ray of the chest showed infiltration of both lung fields. Fever increased. The patient showed resistance to 6-mercaptopurine. His condition gradually deteriorated and he died on the fourth hospital day.

Postmortem examination confirmed the diagnosis of erythroleukemia. The spleen was found to be 400 Gm. The histologic sections showed crowding of pulp and sinuses by erythroblasts and myeloid elements in various stages of maturation. The follicular pattern was, however, preserved. All the other organs were infiltrated by similar type of cells. The heaviest infiltration was found in the lymph nodes, adrenals, liver and heart.

Case 4: Robert F.D. (N.E.C.H. #96–391)

This 29-year-old white male had been in good health until December 1954, when he noticed progressive fatigue. In February 1955, he was observed by us on an ambulatory basis; at that time blood and bone marrow findings led to the diagnosis of acute erythro-
leukemia. The patient was treated with blood transfusions and 100 mg. daily of Meticorten. On May 1955, because of severe weakness and exertional precordial pain, he was admitted to the New England Center Hospital. At this time he presented striking pallor, splenomegaly (5 cm.) and hepatomegaly (8 cm. below the respective costal margin). A flame-shaped hemorrhage was seen in the right fundus. No other physical symptoms were noted.

The hemogram revealed hemoglobin 6.8 Gm. per cent, RBC 2.13 M, WBC 6,300, with 2 myeloblasts. Forty-four nucleated RBC per 100 WBC were seen. Many of them were abnormal in character, with large cytoplasm, nuclear and cytoplasmic vacuoles, immature nuclei. Reticulocytes were 10 per cent and platelets 42,600. On smear the red cells presented many variations in size and shape and macrocytosis was striking. There was also striking stippling and polychromatophilia. The bone marrow aspiration showed increased cellularity with hyperplasia of both the erythroid and granulocytic series. Some nucleated red cells had megaloblastoid appearance. A marked shift to the left of the granulocytes was noted. Total serum bilirubin was 1.6 mg. per cent. No abnormalities were noted in other tests. The red blood cell survival studies showed a slight degree of increased hemolysis. The treatment with Meticorten (20 mg. daily) and blood transfusions was continued.

Following discharge, there was a gradual reduction in the blood counts and an increase in the transfusion requirement. The patient was readmitted to the hospital in June 1955. Since February he had had a total of 10 transfusions, some of which were of packed red cells. The hemoglobin had dropped to 2.6 Gm. per cent, the white blood cells were normal in number, but the myeloblasts had increased to 34 per cent, and 22 per cent of non-well-definable cells with histiocytic features were seen on the smear. The nucleated RBC had decreased to 4 to 8 per cent of WBC. Platelets were 45,000. Reticulocytes were reduced to 2.9 per cent. As before, the red blood cell abnormalities were striking.

The impression was gained that the type of leukemic proliferation was changing into one with definite granulocytic preponderance; the nucleated RBC had decreased in the peripheral blood, while the myeloblasts had progressively increased. The patient was given three blood transfusions and the treatment with Meticorten (20 mg. daily) was continued.

In September 1955, the patient was admitted to the Boston V.A. Hospital. From June to September, the patient had received no blood transfusions. However, the hemoglobin level had dropped considerably. About one month prior to admission the patient had had a few carious teeth extracted and although he did not bleed excessively at that time, the gums failed to heal and became infected, for which he was treated with penicillin.

On admission to the V.A. Hospital, the patient was febrile (100.8°) and remarkably pale. He was however in no acute distress. A few petechiae were seen on the soft palate and on the buccal mucosa. No jaundice was noted. Hepato- and splenomegaly were of the same degree as previously. A few shotty lymph nodes were palpated in the left axilla and in both inguinal regions. The blood examination showed hemoglobin 7.9 Gm. per cent, and hematocrit 25 per cent. The WBC was 32,000 per cu. mm., with a differential of 5 neutrophils, 1 metamyelocyte, 58 lymphocytes, 17 monocytes, 8 myelocytes and 11 myeloblasts—3 nucleated red cells per 100 WBC were seen. Anisocytosis and pochilocytosis were striking. Platelets appeared decreased on smear. The bone marrow examination showed a densely cellular marrow with increased predominance of the granulocytic series. The M/E ratio was 10/1. Many reticulum cells were seen. The impression was gained that the patient had acute leukemia, probably of the myelomonocytic type. The patient was given two blood transfusions after which he felt more comfortable. However, he began complaining of soreness in his throat and in the following days he showed difficulty in breathing. On the fifth hospital day, the patient suddenly died.

At autopsy, laryngeal ulceration, acute edema and respiratory obstruction were found. Pneumonia of the right lower lobe was detected. Spleen, liver and lymph nodes were found enlarged. The spleen was 1000 gr. The malpighian bodies were few and widely separated. There was diffuse infiltration of myeloid cells of blastic type with relatively large nuclei, some of which were indented. The same type of infiltration was found in
ANEMIA OF THE DI GUGLIELMO SYNDROME

the lymph nodes, liver, lungs and other organs. The bone marrow was highly cellular with great preponderance of immature cells of the granulocytic and monocytic series.

Case 5: George M. (N.E.C.H. #103–957)

This 36-year-old white male was first admitted to the New England Center Hospital on April 21, 1956, with a long history of a slowly progressive rheumatoid spondylitis which had begun during early high school years. Between 1950 and 1954 he had received three courses of x-ray treatment over the spine. During the month prior to admission he began to note lightheadedness when changing posture, weakness and fatigue.

On physical examination, there were no symptoms except for a striking pallor. No petechiae or ecchymoses and no lymphadenopathy or hepatosplenomegaly were found. The blood counts showed anemia (7 to 8 Gm. Hgb.) and leukopenia (2,350 WBC) with a normal platelet count. On the smear, there were 4 per cent myeloblasts. Reticulocytes were 1.4 per cent. The red cell morphology was not remarkable. Slight anisocytosis and occasional ovalocytes were noted. The sedimentation rate was 115 mm. Other clinical tests gave normal results.

No nucleated red cells were seen in the peripheral blood at that time; however, the bone marrow showed hypercellularity with preponderance of the more immature red cell precursors, many of them with an atypical nuclear network, histioid and megaloblastoid character, and a large number of mitoses. Myeloblasts and promyelocytes were also increased in number. The diagnosis of acute erythroleukemia was made. Therapy with prednisone (100 mg. daily) and blood transfusions was begun. Injections of vitamin B_{12} had no effect. Transfusions were beneficial and the patient was able to resume work.

During the following 11 months, the blood counts did not change except for a progressively marked leukopenia which reached the value of 1150 WBC. The immature white cells had disappeared from the peripheral blood. The differential count remained within normal limits and no nucleated red cells were seen. The platelet count remained at about 300,000. Reticulocytes were consistently low.

In November 1956, the patient was given a course of 6-mercaptopurine (200 mg. daily) in addition to Meticorten (20 mg. daily). In January 1957, his weakness and fatigue had become more marked and fever started to appear. He was found to have a furuncle on the neck. He was treated with erythromycin and the fever subsided. Up to this time he had never had hemorrhage or purpura. Stool guaiac was always negative. At the end of the same month he had an episode of lower abdominal pain with suprapubic localization and slight fever. This was followed by watery diarrhea which continued a few days after the fever had subsided and there were bloody stools. The liver was now palpable 2 cm. below the costal margin. Spleen and lymph nodes were not enlarged. Total serum bilirubin was 1.9 mg. per cent (1.2 indirect). Reticulocytes were 1.4 per cent.

On March 21 of the same year, the patient was readmitted for incision and drainage of a carbuncle in the right occipital area. At that time he was found to have diabetes, presumably secondary to steroid therapy. His course had been one of increasing deterioration with an increased transfusion requirement. The WBC which always had been low in the past was now found to be 14,400 with immature forms of granulocytes and myeloblasts (12 per cent). Ten per cent nucleated RBC were seen and platelets were markedly reduced on smear. Purpura was present but there was no other bleeding. The patient was suffering from pain in the legs on the lateral aspect and from crampy lower abdominal pain before evacuation. There was also spontaneous pain in the left upper quadrant, probably due to a splenic infarct. Low-grade fever was present. After the incision of the carbuncle the patient was sent home on the same treatment as previously.

On March 28, 1957, the patient was readmitted to the hospital in a terminal stage with purpura and high fever. He died shortly thereafter in peripheral vascular collapse. Post-mortem examination was not obtained.


The patient, a 61-year-old retired army colonel, was referred to our laboratory in February 1957, for further study because of a "refractory anemia" of approximately 10 years' duration.
In 1947 the patient had noticed insidious onset of fatigue and the diagnosis of anemia was made at that time during hospitalization in an army hospital in Japan. Hemoglobin was found to range between 12 and 13 Gm. per cent. The patient remained on active duty for four more years. At the end of this period he was rehospitalized in the United States and was retired from the army with the diagnosis of "iron deficiency anemia." However, hospital records obtained from this period showed no evidence of hypochromia (the RBC averaging 3.75 M with 11.5 Gm. per cent of hemoglobin), and the anemia failed to respond to iron therapy. A bone marrow aspiration done at this time was read as "negative." The smears of the peripheral blood showed anisocytosis, basophilic stippling, and on one occasion a few nucleated red blood cells were noted. The white blood cell count and the differential were within normal limits.

After discharge from the army in 1951, the patient continued to be troubled by fatigue, and in 1955 he was admitted to the Murphy Army Hospital. Physical examination was essentially negative save for pallor. A bone marrow aspiration done at the time was reported to show marked erythroid hyperplasia. The bone marrow was examined repeatedly, with consistent findings of marked hyperplasia of nucleated red cells, although the reticulocyte count in the peripheral blood was low. Anemia became progressively increased and smears of the peripheral blood showed consistently marked anisocytosis, poikilocytosis, polychromatophilia and the presence of nucleated red blood cells. The WBC and differential counts were both normal.

When we first observed the patient in February 1957, marked pallor and slight icterus of the sclerae were present. Neither the liver nor spleen were palpable and there was no peripheral lymphadenopathy. Anemia was very marked (Hgb. 3.9 Gm. per cent). The leukocyte counts ranged between 4,600 and 5,000 per cu.mm. The differential count showed for the first time in the patient's history, an increased number of immature forms including 13 per cent myeloblasts. The nucleated RBC had also increased (18 per cent of the WBC). Megakaryocyte fragments were seen. Platelets were 109,200. The smear showed also marked anisocytosis and poikilocytosis of the red cells. Howell-Jolly bodies were present in some of the red cells. Bone marrow examination showed marked erythroid hyperplasia with proportionately increased number of immature forms, many of which were megaloblastic in character. There was a shift to the left of the granulocytic series.

The number of reticulocytes in the peripheral blood (7.2 per cent), the serum bilirubin (2.8 mg. per cent) and the fecal urobilinogen (1051 mg. per day) all pointed to the presence of increased hemolysis. The Cr\(^{51}\) red blood cell study showed a somewhat diminished red cell life span (19 days of T \(\frac{1}{2}\) Cr\(^{51}\)); however, the degree of hemolysis did not correlate with either the marked degree of red cell hyperplasia seen in the marrow or the severe degree of anemia. The Coombs' test was negative. The sedimentation rate was 71 mm./hr. (Westergren); all other laboratory tests gave normal results.

This patient with a 10-year history of a normochromic, normocytic anemia with increased cellularity of the bone marrow, developed nucleated red blood cells in the peripheral blood about 6 years after onset of anemia. At this time, the presence of erythroblasts and myeloblasts in the peripheral blood, the increased cellularity of the bone marrow, the shift to the left of the granulocytic series and the megaloblastoid appearance of the normoblasts were all indicative of the diagnosis of chronic erythroleukemia.

Follow-up studies during the past year revealed no basic changes. Transfusions were required averaging 200 cc. per month. In the last two months of 1957, the transfusion requirement increased considerably and in December the patient received one unit of blood a week. He had also been treated with 60 mg. daily of prednisolone.

On January 1, 1958, the patient was transferred to the Boston U.S. Public Health Service Hospital. On admission it was found that the patient's condition was poor and had deteriorated rapidly. He had lost about 30 pounds in weight during the preceding few months. He was suffering from insomnia, easy fatigability, weakness and an unproductive cough. On physical examination the patient showed intense pallor but no jaundice. There was no fever and the lungs were clear to percussion and auscultation. There was an apical systolic murmur. Liver and spleen were not enlarged. There were discrete, non-tender axillary nodes. No petechiae or hemorrhagic spots were seen. The
laboratory data showed hemoglobin 6 Gm. per cent, WBC 5,225 with 52 per cent polymorphonuclear leukocytes, 13 per cent band forms, 34 per cent lymphocytes, 1 per cent monocytes. There were 43 nucleated RBC per 100 WBC. Serum bilirubin was normal. The treatment with prednisolone (60 mg. daily) was continued. The patient was also given a course of Myleran (4 mg. daily for 40 days) without any significant response. He later developed recurrent pulmonary infections, severe bronchitis and bronchopneumonia for which he was repeatedly treated with antibiotics. Anemia and thrombocytopenia remained unchanged during the hospital course, in spite of frequent blood transfusions and of the administration of steroids. In May 1958, the patient developed severe bronchopneumonia which did not respond to antibiotic therapy. At this time he showed widespread and generalized petechiae. On May 26, 1958, the patient expired. Tissues removed at autopsy showed multiple tumor formations in several organs, but particularly frequent in the liver. They were composed of malignant reticulum cells and immature cells of erythroblastic and myeloblastic series. Smaller infiltrates of reticulum, myeloid and erythroid cells were found in the lymph nodes particularly of the periaortic area. Smaller lymph nodes were more leukemic in appearance with infiltration of cells more than organoid formation. Other findings were not remarkable. A similar case of erythremic myelosis, with tumor-like formations of malignant reticulum cells as well as erythroleukemic infiltration, was described by Martin, W. J. and Bayrd, E. B.4

Case 7: Charles S.H. (N.E.C.H. #57-60 Lab.)

This 70-year-old business man was seen in the Blood Research Laboratory of the New England Center Hospital on January 20, 1957, as an out-patient. He complained of easy fatigability, weakness and pallor, and said he had lost 30 pounds in the past 4 years. There was no history of serious illness or operations. In October 1955, the patient had pneumonia which cleared with antibiotics, but one month prior to the onset of the pneumonia he noted gradual weakening, increased fatigue and pallor. Anemia of 1.5 million RBC was discovered and a few blood transfusions were given. The anemia as defined by the RBC and Hgb. indices was of an unusual type: macrocytic and hypochromic with MCV 125 cu. micro, MCH 20.5 micro-micrograms, and MCHC 25 per cent. There was leukopenia (3,440 WBC per cu. mm.) with neutropenia; 388 nucleated RBC per 100 WBC were noted on the smear. Reticulocytes were 4.5 per cent and platelets 93,300. There had been, however, no bleeding or bruising. A bone marrow examination showed an increased number of normoblasts with a myeloid-erythroid ratio of 1.26 to 1 and with many mitotic figures. The indirect bilirubin was 1 mg. per cent. The Coombs' test was negative. Other laboratory tests gave normal results. Treatment with iron, liver extract, vitamin B12 and steroids were without success.

On physical examination, the patient presented a sallow pallor hut appeared in fairly good condition. No icterus was seen. No petechiae, purpura or ecchymoses were found. Lymph nodes and spleen were not enlarged. The liver was palpable 5 cm. below the costal margin.

Due to the large number of blood transfusions recently received (averaging one every two weeks), the RBC count done in our laboratory was higher than had been previously observed. The RBC was 3.36 M, hematocrit 34 per cent and hemoglobin 9.7 per cent. The WBC count was 1,816 (corrected) with 68 nucleated red blood cells per 100 white cells. The differential count was normal, but several megalakaryocyte fragments were seen. There was marked anisocytosis and poikilocytosis of the red blood cells, and several target cells were seen. Occasional polychromatophilia and stippling were noted. Reticulocytes were 11.3 per cent and platelets were 292,000. Serum bilirubin was 1.2 mg. per cent. The Coombs' test was negative. Plasma hemoglobin was 12 mg. per cent. Serum iron was 220 µg. per cent. The red blood cell life span determined with the Cr51 method gave a T½ of 15 days (normal 28.1 days). The bone marrow preparation showed hypercellularity with marked erythroid hyperplasia, the M/E ratio being 1:3. The nucleated red cells showed a striking degree of immaturity and an unusual number of erythrogones. Many of the normoblasts contained broken, fragmented and clover-leaf nuclei. Mitoses were numerous. Macronormoblasts were present. The granulocytic series showed a shift
to the left with promyelocytes and myelocytes as the predominant cells. Megakaryocytes were adequate in number. There were many iron granules. The bone marrow findings were consistent with the diagnosis of a myeloproliferative disorder involving both the myeloid and the erythroid series, i.e., *erythroleukemia, chronic in type.*

**Case 8: Harold J.P. (#110–977)**

This 65-year-old white male was admitted to the New England Center Hospital on March 13, 1957, with the chief complaints of weakness, fatigue and anemia. He had been in apparently good health until August 1956, when he began to experience shortness of breath, cough and marked fatigue. There was also some transient ankle swelling. He was seen by his physician and was treated with iron pills and liver injections with no apparent effect. In December he had an onset of fever and severe cough, diagnosed as “virus pneumonia” and treated with antibiotics. The patient improved and fever disappeared. The weakness continued, however, and shortly afterwards he noticed small petechial hemorrhages and also developed two severe epistaxes. On examination the patient was told by his physician that he had leukemia. He was then admitted to his local hospital and treated with transfusions (5 units in 3 months). The patient lost about 20 pounds though his appetite remained good. His chief complaint was weakness which made it impossible for him to continue with his daily activities. There was no history of exposure to x-ray or toxic chemicals.

On examination at his first admission to our hospital, the patient was found to be pale although still in good physical condition. There were petechiae on his wrists and ankles and over both lower quadrants. Scattered petechiae were found in the buccal mucosa and on the tongue. There were 2 to 3 mm. ulcerations on both sides of the nasal septum with eschars, and a mild icterus of the sclerae. No lymphadenopathy was found. Spleen and liver were not palpable.

On laboratory examination, the hemoglobin was 8.1 Gm., hematocrit 29 per cent, RBC 2.54 M, platelets 30,000 and reticulocytes were 3.4 per cent. The WBC was 1,200 with 18 polymorphonuclear neutrophils, 68 lymphocytes, 2 monocytes, 12 myeloblasts. Several nucleated red blood cells were seen; some of these were large and showed asynchronism of nuclear-cytoplasmic maturation. There was marked anisocytosis and poikilocytosis of red cells and fairly pronounced macrocytosis. Serum bilirubin was 1.6 mg. per cent (1.4 of the indirect variety). The Coombs’ test was negative.

The bone marrow was hyperplastic with a marked increase in the erythroid series. There were many giant atypical erythroblasts, often with two to five nuclei and many nucleoli. Many of the immature forms had vacuolated cytoplasms. Some of the more mature normoblasts were megaloblastic, also containing two to three nuclei. There were many mitotic nuclei. Many small orthochromatic normoblasts were also seen. The megakaryocytes were greatly reduced and most were megakaryoblasts. The granulocytic series showed a partial maturation arrest at the myelocyte level. The preponderance of cells were progranulocytes. Blasts were increased in number.

A diagnosis of *acute erythroleukemia* was made. The patient was given transfusions as a supportive measure and was started on Meticorten, 25 mg. daily.

Soon after discharge the patient developed marked aching in all extremities and tenderness, particularly over the lips. This was followed by chills, fever, sweats and pronounced cough. The patient was treated with penicillin which produced only a slight improvement. The patient also had a profuse epistaxis. After a few days an extensive cellulitis developed over his left arm.

On his second admission on April 4, 1957, the patient appeared acutely ill. He was febrile and dyspneic at rest and had a severe cough. X-ray of the chest showed “pneumonia” of the left lung. There was slight icterus of the sclerae. No ecchymoses or petechiae were seen. The liver was 4 cm. below the costal margin. The spleen and lymph nodes were not palpable. The anemia was greatly increased (4.8 Gm. per cent hemoglobin), and leukopenia and thrombocytopenia were of a range similar to that recorded at his previous admission. Several nucleated RBC were still present on smear and a few myeloblasts were seen.
The patient showed little improvement and his situation became gradually worse. He died a few weeks later. Postmortem examination was not obtained.

The patient, a 47-year-old laborer, was admitted to the New England Center Hospital on April 10, 1957, with the chief complaint of severe anemia. There was a history of fatigue, weakness, night sweats and exertional dyspnea beginning in 1953, although the patient was able to perform his work until six to eight months before admission. In 1954, when symptoms had become rather marked, the patient was seen by a physician who performed a bone marrow aspiration which was interpreted as a “P.A.-like picture.” However, free hydrochloric acid in the stomach was found and no papillary changes of the tongue, paresthesias or neurological symptoms were noted. The patient was treated with liver injections and experienced a slight but definite improvement. Since the patient’s condition did not continue to show satisfactory improvement he was hospitalized in 1955 and given several transfusions. After a long course of liver injections, treatment was changed to vitamin B₁₂ and folic acid, and oral iron, none of which had beneficial effect. Transfusions were the only treatment which improved the patient’s condition. The average transfusion requirement was two pints of blood every three weeks.

On admission the patient appeared chronically ill, having lost 30 pounds in the last few months. He showed a dark grayish pigmentation of the skin without overt icterus. No ecchymosis or petechiae were found. Small lymph nodes were seen in the right posterior cervical chain. The liver edge was not felt. The spleen was enlarged 6 cm. below the costal margin. The hemoglobin was 8.1 Gm. per cent with hematocrit of 28 per cent. The WBC count was 11,700 and on the smear there were 2 per cent myeloblasts, 0.8 per cent promyelocytes, 0.4 per cent metamyelocytes and 25 per cent band forms. No nucleated red cells were seen. Platelets were 433,000 and the reticulocytes 2.8 per cent. There was marked anisocytosis, poikilocytosis and macrocytosis of the red cells. Microcytes were also present. No spherocytes were seen. The Coombs’ test was negative and plasma hemoglobin was 9 mg. per cent. Serum iron was reported as 189 gammas per cent. Total bilirubin was 1 mg. per cent. All other tests performed gave normal results.

The bone marrow was strikingly hyperplastic and one-half of the cells were erythroid elements, some with megaloblastoid, others with macroblastic features. The number of erythroblasts and the number of mitoses were increased. The myeloid series showed a marked shift to the left with 2.8 per cent myeloblasts and 5.2 per cent promyelocytes.

The peripheral blood picture was one of a mild chronic granulocytic leukemia with a corresponding white cell immaturity in the bone marrow, associated with a marked erythroid hyperplasia with abnormality of maturation and reproduction of these cells. The absence of symptoms of overt hemolysis, the megaloblastic character of the erythroblasts and the lack of response to vitamin B₁₂ treatment led us to the conclusion of a myeloproliferative disorder involving both red and white cells and of a chronic variety, i.e., chronic erythroleukemia.

During hospitalization the patient received two blood transfusions. After discharge (April 19, 1955) the patient was treated for two months with 6 mg./day of Myleran without any evidence of improvement. During this period, 14 blood transfusions were given. In July, a treatment with prednisone was instituted which seemed to lower the transfusion requirement. Later, however, no further beneficial effect was obtained and the course was one of progressive deterioration with frequent infections and an increasing need for transfusions. The patient died on December 7, 1955. At the autopsy, bilateral pneumonia and an abscess of the right lower lobe were found. Hemochromatosis and cirrhosis of the liver were seen. All organs, particularly spleen and liver, were infiltrated with erythroid and myeloid cells and an enormous number of megakaryocytes in all stages of maturation.

The patient, a 56-year-old retired rear-admiral, was admitted to our hospital on October 11, 1955, for evaluation of a chronic, slowly progressive, ill-defined anemia of ap-
proximately 4 years' duration. Prior to and following this admission he was studied at the U.S. Navy Hospital at Portsmouth, Virginia and also by several consultant hematologists.*

In 1951 the patient's hemoglobin was 11 Gm. per cent; in 1953 it was 9 Gm. In January 1954, when hemoglobin was found to be 7 Gm. per cent, vitamin B₁₂ and iron were given with no effect. In May 1954, the patient was admitted to the Bethesda Naval Hospital and the blood counts showed a marked degree of anemia with hemoglobin of 5.7 Gm. and a RBC count of 1.27 M. Numerous studies were performed and many opinions given at that time, but no definite diagnosis was made. The bone marrow biopsy was reported to show "increased cellularity." The patient was treated with 100 mg. of cortisone daily without appreciable effect. Starting in June 1954, transfusions were given with an average transfusion requirement of approximately 25 cc. per day.

At the time of admission to the New England Center Hospital, the patient appeared well. He was afebrile and no obvious pallor or icterus were found. There was no lymphadenopathy. The liver extended 5 cm. below the costal margin, but the spleen was not palpable. The remainder of the physical examination was negative. No petechiae or ecchymoses were seen.

Because of the recent transfusions, the patient's blood counts were improved. Hemoglobin was 8.4 Gm., RBC 2.38 M, and WBC 8,000 with 55 per cent polymorphonuclear neutrophils, 17 lymphocytes, 26 monocytes, 2 basophils. Reticulocytes were 1 per cent, platelets 470,000. There was striking anisocytosis and poikilocytosis, and some stipple cells were seen. The sedimentation rate (Westergren) was 12 mm./hr. Liver biopsy showed minimal portal fibrosis and increased amounts of hemosiderin. Other tests, including the liver function tests and x-rays were all within normal limits.

Bone marrow aspiration confirmed the presence of increased cellularity with hyperplasia of all cell series. There was an increased number of erythroblasts, some having a definite megaloblastoid appearance. The more mature forms were macronormoblastic. There was also a marked increase of reticulum cells. The granulocytic series showed many early forms. The myelocytes were atypical with immature nuclei and diminished granularity. Megakaryocytes were also increased in number.

It was concluded that the patient had an atypical type of leukemia involving the white as well as the red cell series, i.e., a chronic type of erythroleukemia.

Follow-up of the patient during the past two years confirmed this diagnosis. Bone marrow examination was repeated, and the erythroid hyperplasia was found to be highly increased and primarily megaloblastic in type. The white cell series showed an increasing number of myeloblasts. In the peripheral blood the leukocyte number remained normal and no myeloblasts appeared. However, a few nucleated red cells and fragments of megakaryocytes were seen on several occasions. Monocytes increased progressively and were 32 per cent on the last examination.

The patient has progressively lost weight, although the other physical findings have shown little change. The transfusion requirement has remained at one transfusion every 15 to 21 days. The only slightly increased rate of hemolysis has been constant. The red cell life span determined with the Cr³⁺-labeling technic was 22 days of T²¹ Cr³⁺ in 1955, and when repeated in 1957 with the N⁴ γ-glycine technic, was 80 days of mean cell life. This was consistent with the constantly low reticulocyte level in the peripheral blood (under 1.5 per cent) and the normal serum bilirubin. However, in each of several determinations fecal urobilinogen was greatly increased (up to 459 mg. per day).

Several other attempts of treatment with vitamin B₁₂, folic acid, vitamin C and crude liver extracts were always unsuccessful. Cortisone was continued at 75 to 100 mg. levels and has only recently been reduced to 45 to 60 mg. per day.

*We wish to acknowledge with thanks the cooperation of Dr. Watson James, III, Associate Professor of Medicine at the Medical College of Virginia, who supplied many data for the study of this patient.
ANEMIA OF THE DI GUGLIELMO SYNDROME

Case 11: John S. McD (N.E.C.H. #116-051)

This 59-year-old pediatrician was admitted to the New England Center Hospital on November 13, 1957. The patient's history revealed that he had been in good health until nine months before admission when he first noted fatigue, irritability, intermittent cough and wheezes. A blood examination performed one month before this time had revealed a normal hematocrit value. For six months after symptoms appeared, he was able to engage in all his normal activities. After this time his respiratory symptoms became worse with cough and fever. A chest film showed peribronchial infiltration. The patient was first treated with Chloromycetin, Achromycin and Gantrisin. When these gave no relief, he was successfully treated with Furadantin and Comicin. The red blood cell count at that time was of 3.8 M and the white blood cells were 4,800 with 75 per cent lymphocytes. Bone marrow examination showed a "hyperactive" marrow. Two months later when the patient entered the Oak Park Hospital in Chicago, it was found that the anemia and leukopenia had progressed. Fatigue with dyspnea on exertion had also become prominent. However, there had been no significant weight loss, no jaundice, no hepato- or splenomegaly, no lymphadenopathy.

On admission to the New England Center Hospital, the patient showed marked pallor but was not in acute distress. There was an ecchymosis on the left medial forearm. Retinal examination showed a large hemorrhage of the left fundus. Smaller hemorrhages adjacent to the vessels were seen in both fundi. There was no scleral icterus. No pulmonary abnormalities were found. Liver and spleen were normal. No lymphadenopathy was seen.

Laboratory data revealed: hemoglobin of 7 Gm. per cent, hematocrit 20 per cent, red blood cells 2.7 M. Platelets were 166,000 and reticulocytes 0.8 per cent. White blood cells were 2,700 with lymphocytes predominating. Two per cent myelocytes and 2 normoblasts per 100 WBC were noted. Anisocytosis and poikilocytosis were marked and many target cells were seen. The Coombs' test was negative. Serum iron was 270 gamma per cent. Total serum bilirubin was 0.8 mg. per cent. Blood sedimentation rate was increased. All other tests gave normal results. A bone marrow examination showed striking erythroid hyperplasia with large numbers of megaloblasts. There were frequent mitoses of erythroblasts, and some cells were multinucleated with 3 to 6 nuclei. Asynchronism of nuclear-cytoplasmic maturation was present in many cells. The white cell series and megakaryocytes were normal. The serum vitamin B₁₂ level was increased (2544 μg per ml.).

On the basis of these findings, the diagnosis of erythremic myelosis, subacute in type, was made. Therapy with vitamin B₁₂ and folic acid, given on the basis of the intense megaloblastosis, was unsuccessful. The patient was then treated with blood transfusions and has required an average of two transfusions per month.

REFERENCES


The Anemia of the Di Guglielmo Syndrome

MARIO BALDINI, HUGH H. FUDEMBERG, KATSUMIRO FUKUTAKE, WILLIAM DAMESHEK and Angela Pasquarriello