Dyserythropoiesis, Refractory Anemia, and "Preleukemia?" Metabolic Features of the Erythrocytes

By William N. Valentine, Patricia N. Konrad, and Donald E. Paglia

Twenty-one enzymatic activities and red cell glutathione content are compared in cord blood, dyserythropoietic disorders, normal subjects, and subjects with hemolytic syndromes and reticulocytosis approximating that of the newborn. The dyserythropoietic states are heterogeneous and include several hereditary disorders, "preleukemia" and overt leukemia, refractory anemia with and without marrow ringed sideroblasts, and folate deficiency. Many activities exceeded those of cord erythrocytes and "high reticulocyte" controls by one or even several standard deviations. The low phosphoglycerate kinase and glutathione peroxidase and relatively low ribosephosphate pyrophosphokinase and adenine-phosphoribosyl transferase of cord erythrocytes were mimicked uncommonly. Pyruvate kinase was often relatively or absolutely low and sometimes dramatically so. Enzyme ratios were grossly aberrant. Capricious very high individual activities occurred in some instances. The heterogeneous nature of case material, including both hereditary and acquired syndromes, renders it difficult to interpret similarities often seen from case to case and sometimes to cord blood patterns on the basis of the reversion to fetal erythropoiesis as a common denominator. Rather, the cyto- and karyokinetic abnormalities characterizing dyserythropoiesis of diverse etiologies may result in exaggerations and distortions of a normal asynchronism in loss of functional genetic material governing enzyme synthesis as the nucleus degenerates and cytoplasmic organelles are lost.

The dyserythropoietic anemias are clinically, hematologically, and metabolically heterogeneous. They may be congenital or acquired, responsive to pyridoxine and androgens, and may or may not evolve into overt myeloblastic leukemia. Ringed sideroblasts may be abundant, inconspicuous, or absent in the bone marrow. The marrow morphology in some instances has special characteristics, such as erythroid multinuclearity, megaloblastoid features, or other evidence of dysplasia. The peripheral blood frequently contains a variably numerous population of hypochromic erythrocytes together with well-hemoglobinated cells. Oval macrocytes are prominent in some cases; in others, shape changes of the

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erythrocytes are inconspicuous. In still other cases, bizarre morphology and marked poikilo- and anisocytosis characterize the stained blood film.

The dyserythropoietic states share certain features, however. In most states there is marrow erythroid hyperplasia, and erythropoiesis is ineffective. The ineffective erythropoiesis is characterized by evidence of intramedullary cell death and abnormally large bile pigment excretion not attributable to destruction of circulating red cells. The reticulocyte count is often inappropriately low in relation to the degree of anemia and marrow hyperplasia. Ferrokinetic studies most frequently show increased plasma iron turnover with defective utilization of iron. Red cell lifespan may approach normal, or variably severe hemolysis may complicate defective erythrocyte production. Leukocytes and platelets may or may not be involved in the disturbed hematopoiesis. In some, but by no means all instances, chromosomal abnormalities have been noted.

These hematologic aberrations have metabolic counterparts. There is often evidence of defective synthesis of heme. Hb F is sometimes modestly (or occasionally markedly) increased. There are other resemblances to fetal erythropoiesis. These include a glycine γ-chain/alanine γ-chain ratio like that in cord blood, an increase in the i antigen, a pattern of lactate dehydrogenase (LDH) isozyme distribution similar to that of cord blood, and alterations in red cell enzyme activities sometimes resembling those of the newborn. A decrease or absence of antigens A1, H, and B is sometimes noted. In addition, low red cell pyruvate kinase activity has frequently been observed, although this is not invariable. Several separate clones of cells have been identified in the erythrocyte population of some subjects.

Extensive investigations, particularly in France, have emphasized the diverse nature of enzymatic and serologic abnormalities in idiopathic refractory anemias and in the dyserythropoiesis associated with some leukemias. While recognizing that embryonal characteristics are varied and inconstant and that features of adult erythropoiesis are also present, Rochant et al. have suggested that reactivation of normally repressed fetal genes could possibly be related to the etiology and to the malignant transformations sometimes seen in the dyserythropoietic states. The production of embryonal proteins in certain leukemic and other malignant states is well documented.

The present report presents a comparison of the erythrocyte glutathione (GSH) content and 21 enzymatic activities in dyserythropoietic disorders of diverse etiology with those of erythrocytes of normal subjects, with those of cord blood, and with those of selected subjects with hemolytic anemia and reticulocytosis approximating that normally present in the newborn.

CASE REPORTS

The case material can be arbitrarily divided into four groups: (A) heterogeneous group of congenital dyserythropoietic states; (B) cases considered as probably "preleukemic" or who have developed overt leukemia; (C) acquired idiopathic refractory anemias with and without ringed sideroblasts in the marrow; (D) a single case of severe megaloblastic...
anemia due to folate deficiency. Since the case material is voluminous (28 subjects),
descriptive data of necessity are limited. Certain pertinent information is summarized in
Table 1. No subject had a positive antiglobulin test, an abnormal hemoglobin, or
evidence of paroxysmal nocturnal hemoglobinuria.

A. Congenital or Probably Congenital Dyserythropoietic Disorders

Cases 1 and 2, A.P. and R.P., are brothers, the offspring of a first cousin marriage,
and have been anemic since birth. The disorder has been characterized by Hb values in
the range of 9–10 g/100 ml, mild neutropenia, normal platelets, and reticulocytosis of
1%–6%. The peripheral blood film shows marked poikilo- and anisocytosis, elliptocytes,
burr cells, teardrop forms, and schistocytes. The bone marrow aspirate reveals intense
erthropoiesis with about 5% binucleate erythroblasts. The nuclei of the latter
characteristically vary in size and depth of staining, and in some, karyolysis and
karyorrhexis are prominent. Internuclear chromatin bridges are rare but present. The
anemia conforms to that described by Wendt and Heimpe13,14 as congenital dyserythro-
poietic anemia (CDA) Type I. Both parents have normal hemograms and enzymologic
studies.

Cases 3, 4, and 5 have hereditary erythroblastic multinuclearity associated with a positive
acidified-serum test (HEMPS,9 CDA Type II10). They have previously been reported
in detail (cases 1, 4, and 5;9 cases M.F., L.F., and C.L.47). The disorder is characterized
by erythroblastic multinuclearity, ineffective erythropoiesis, a positive acidified-serum test
having different characteristics from the Ham test, and by unusual susceptibility of the
red cells to agglutination with anti-i and lysis by anti-i and anti-I.9

Cases 6, 7, 8, and 9 have many similarities, but identity is not proved. Cases 6 and 7,
J.V. and C.V., are sisters, and have been anemic since birth. While Hb values have often
been in the range of 5–7 g/100 ml, transfusions have been rare. Erythrocyte morphology
has been characterized by marked aniso- and poikilocytosis, occasional teardrop and
fragmented forms, a number of oval macrocytes, and a small population of hypochromic
cells. Reticulocyte and platelet counts are normal, and slight neutropenia is present. The
marrow shows erythroid hyperplasia and mild megaloblastoid features. Chromosomal
karyotypes derived from marrow culture (case 6) are normal. The 51Cr red cell lifespan
is shortened (Table 1), and there is evidence of increased body iron stores. There has been
no response to any form of hematinsics. The hemogram of both parents is normal, as are
the enzymatic activities of their erythrocytes. No consanguinity is known in the kindred.

Case 8, K.Y., a female of Japanese descent, has had recognized anemia since her early
teens. The anemia probably is congenital, although four siblings are said to be normal.
The peripheral blood morphology is essentially identical to cases 6 and 7. The marrow
exhibits marked erythroid hyperplasia (M/E = approximately 0.7/1.0) and some
megaloblastoid features. Both neutropenia and thrombocytopenia have been present, and
the reticulocyte count has always been inappropriately low. The anemia has improved
slightly on androgens, folate, and pyridoxine therapy but still persists together with
leukopenia. Chromosome studies based on peripheral blood cultures have shown no
significant abnormalities. The parents are not known to be consanguineous and have no
detectable abnormalities of red cell metabolism or of their hemogram.

Case 9, M.F., is a 21-yr-old male and has been followed at the University of California,
Los Angeles since age 10. The refractory anemia has responded very partially to high-dose
androgen and pyridoxine therapy. The peripheral blood cell morphology resembles
closely that of cases 6, 7, and 8. Pancytopenia and marrow erythroid hyperplasia have
consistently been present. Reticulocytosis has never been commensurate with the anemia.
Cytogenetic studies on both marrow and peripheral blood cultures have shown no
significant chromosomal abnormalities. While both parents are well, an older brother and
two cousins of the patient have died of idiopathic aplastic anemia, one recently in the
UCLA hospital. While not fully proved, the patient's refractory anemia is considered
as probably congenital.

Cases 10, 11, and 12 are of diverse etiology but are believed to be congenital. Case 10,
C.J., a 13-yr-old girl, was noted to be anemic at age 3. By age 6 she had received 30 pints of transfused blood. The Hb has usually ranged from 4.5 to 7.5 g/100 ml, the reticulocytes have often been inappropriately low, and moderate to mild leukopenia with normal platelets has been noted. The bone marrow shows increases in stainable iron and a scattering of ringed sideroblasts. The peripheral blood morphology is bizarre with aniso-, poikilo-, and microcytosis, and hypochromia. There has been a very partial response to pyridoxine therapy chiefly in the form of increased reticulocytosis. The patient is considered to have a congenital refractory anemia of undetermined type.

Case 11, M.H., a 26-yr-old female, was found to be anemic at age 15. The spleen was enlarged and was removed in 1965 without improvement in the anemia. The peripheral blood exhibits aniso- and poikilocytosis, target cells, some macrocytosis, and some teardrop forms and schistocytes. The marrow shows low normal to mild hypocellularity. The M/E ratio is 1.5/1.0. Modest reticulocytosis and moderate shortening of erythrocyte lifespan are present.

Case 12, A.C., a 5-yr-old Mexican female, has classical Fanconi’s anemia with absent thumbs, pancytopenia, a hypoplastic marrow, and numerous chromatid breaks and gaps evident on cytogenetic studies based on a marrow culture.

B. Subjects Who Are Probably “Preleukemic” or Who Have Developed Overt Leukemia

Case 13, S.P., an Italian male with incidental beta thalassemia minor, developed severe refractory anemia in 1969. Frank myeloblastic leukemia subsequently evolved. The peripheral blood film demonstrated moderate hypochromia and microcytosis, marked aniso- and poikilocytosis, microspherocytes, increased basophilic stippling, and up to 8 nucleated red cells/100 leukocytes. The picture was much more bizarre than could be attributed to thalassemia minor alone. The marrow was initially interpreted as showing profound erythroid hyperplasia. Of great interest was the consistent presence of an extra “C”-group chromosome in more than 50% of mitoses in cultured marrow.22

Case 14, A.K., a 55-yr-old white female, was found to be anemic on routine examination in 1969. This was progressive and accompanied by severe neutropenia and thrombocytopenia. The marrow showed marked erythroid hyperplasia. Splenectomy was performed without improvement. Late in the disease, the marrow became infiltrated with immature granulocytes; the leukocyte count rose to high levels with large numbers of myeloblasts, and up to 88 nucleated red cells/100 leukocytes were noted. Cytogenetic studies based on cultured marrow showed no significant chromosomal abnormality. Enzyme studies were performed during the period of pancytopenia and refractory anemia.

Case 15, N.W., a 41-yr-old black male, was found to have mild anemia, thrombocytopenia, a leukocyte count of 1700/cu mm, and four nucleated erythrocytes/100 leukocytes at the time of routine examination in 1971. Since then he has had five hospitalizations with icterus, hemolysis with periodically severe exacerbations, and anemia requiring repeated transfusions. The peripheral blood has shown from 1000 to 14,000 nucleated red cells/100 leukocytes and reticulocytosis at times as high as 40%. Marrow erythroid hyperplasia without dysplasia is present. Cytogenetic studies have revealed no chromosomal abnormalities. In ferrokinetic studies, maximum iron incorporation was but 20% at 2 days, and there was rapid plasma clearance of 59Fe. The peripheral blood consistently shows 2%–3% myeloblasts. Incipient myeloblastic leukemia or a variant of erythremic myelosis with marked hemolysis are the working diagnoses.

Case 16, H.H., a 61-yr-old white male, in late 1969 developed progressive fatigue. In March 1970, pancytopenia, mild splenomegaly, and marrow erythroid hyperplasia with megaloblastoid changes were observed. A small number of blasts were present in the peripheral blood. Pancytopenia probably due to “preleukemia” was diagnosed. On pyridoxine, folate, prednisone, and iron the reticulocytes initially increased, and the PCV rose from 28% to 32%. Undifferentiated leukocytes and thrombocytopenia remained. The patient expired in August 1970 with massive pneumonia due to Aspergillus and Candida. At autopsy there were also Aspergillus abscesses of the right frontal lobe of the brain and
left thyroid, a single microscopic metastatic adenosacrinoma of a carinal lymph node without any primary site being found, and a simply hyperplastic marrow. Neutrophils also were shown to lack myeloperoxidase. Incipient leukemia could not be documented with certainty as an explanation of the 9-mo period of refractory anemia.

Case 17, H.K., a 46-yr-old white male, presented with acute myeloblastic leukemia in April 1971. Following two courses of cytosine arabinoside and 6-thioguanine, complete remission was obtained in June 1971. This has since been maintained on a somewhat unusual program of near weekly intravenous cytosine arabinoside supplemented at times with oral 6-thioguanine.

C. Acquired Refractory Anemias

Case 18, V.U., a 79-yr-old white nurse with inactive rheumatoid arthritis, was referred by the Hematology Service of the University of Utah where a diagnosis of refractory anemia with ringed sideroblasts had been established. She exhibits well-marked anemia, modest leukopenia, and a cellular marrow with 10% ringed sideroblasts. No response to 100 mg of pyridoxine daily has been achieved.

Case 19, E.Z., a 46-yr-old white female, presented in 1969 with severe pancytopenia and a slightly hypocellular marrow with erythroid preponderance (M/E = ≈ 0.14). She responded to large doses of corticosteroids with return of all elements to near normal values and development of a hypercellular marrow. On continuing medication, the PCV fell to 19%, and pancytopenia recurred. Since renewing steroid therapy, reticulocytosis to 16% has developed, but anemia persists. There now is evidence of red cell sequestration by an enlarged spleen. Enzyme studies were performed during relapse when the patient had received no therapy for several months.

Case 20, A.S., an 81-yr-old white female, has severe anemia, very mild leukopenia, slightly reduced platelets, and an inappropriately low reticulocyte count discovered during a recent hospitalization. Marrow shows intense erythroid hyperplasia. The peripheral blood exhibits moderate anisocytosis and polychromasia and marked basophilic stippling. The serum haptoglobin is moderately reduced.

Case 21, H.F., a 64-yr-old white male, had normal blood counts documented in 1966. In 1970, anemia without leukopenia or thrombocytopenia was first noted. The marrow was hyperplastic and contained increased numbers of ringed sideroblasts. No underlying disease has evolved, and therapy with various combinations of pyridoxine, androgens, and folate has failed to effect improvement. In ferrokinetic studies, maximum incorporation of 59Fe was but 48%, achieved on day 12 after injection.

Case 22, A.F., a 73-yr-old black female, was diagnosed as having refractory anemia in 1970. Anemia was severe, while leukocyte and platelet counts were normal. The stained peripheral blood film shows macroovalocytes, anisocytosis, and poikilocytosis. The marrow exhibits marked erythroid hyperplasia. No increase in blasts or foreign cells were observed. No underlying disease has thus far been apparent.

Case 23, R.F., a 70-yr-old white male, has been anemic since June 1971. There has been no response to hematinics. The leukocytes are normal. The peripheral blood exhibits poikilocytosis, a considerable number of oval macrocytes, and scattered, distorted, hypochromic red cells. The platelets are greatly increased. Some transfused cells were present in the patient's blood at the time of our studies.

Case 24, R.W., a 62-yr-old white female, was discovered to be pancytopenic in 1971. The peripheral blood shows aniso- and poikilocytosis, while marrow aspirates are cellular with an M/E ratio of 0.14/1.0. Mild megaloblastoid features are present. The patient has responded with reticulocytosis up to 16% on androgen therapy, but the PCV has remained 28%–31%. The patient has refractory anemia and pancytopenia without evidence of leukemia at this time.

In Case 25 (E.H.) refractory anemia, mild leukopenia, and a normal platelet count were found during hospitalization for cardiovascular evaluation in June 1972. The peripheral
blood exhibited moderate aniso- and poikilocytosis and normochromic red cells. The marrow showed moderate erythroid hyperplasia and abundant iron. All other studies have been normal.

Case 26, M.R., a 73-yr-old white male, was found to be severely anemic and pancytopenic at the time of hospital admission for cardiovascular studies. The red cells were normochromic and normocytic with little variation in size and shape. The marrow was hyperplastic, but the erythroid series was relatively normal in appearance. The granulocytic series was hyperplastic with some diminution in later forms. No response to hematins has been obtained, and no underlying disease has thus far appeared.

Case 27, V.L., a 55-yr-old white female, was found to be anemic in early 1972. The peripheral blood showed macrocytosis, aniso- and poikilocytosis, a few teardrop cells, and normal leukocytes and platelets. The serum iron was increased to 200 µg/100 ml. A bone marrow aspirate was hypercellular with erythroid preponderance (M/E = 1/1), and some megaloblastoid features. The patient subsequently failed to respond to either B12 or folate.

D. Folate Deficiency

Case 28, R.K., a 51-yr-old white female alcoholic, presented with marked anemia. Dietary history indicated poor nutrition. The peripheral blood contained many large oval macrocytes, and aniso- and poikilocytosis were present. Platelets were adequate, and there was a mild increase in hypersegmented neutrophils. The marrow was frankly megaloblastic. The serum iron was 250 µg/100 ml. Free gastric acid was present. The administration of 1 mg of folate daily resulted in prompt reticulocytosis to 35% and a rising PCV. At the time of study, therapy had been started a few days previously, and reticulocytosis of 25% was present.

E. Cord Blood

Cord blood samples were obtained from 22 normal, full-term infants. The mean PCV was 48.2 ± 4.5 ml/100 ml of blood. The mean reticulocyte count was 3.8% ± 1.0% with a range of 2.3%–7.0%.

F. Normal and Reticulocyte-rich Control Blood.

Subjects serving as normal controls were clinically well and had normal hemograms and reticulocyte counts. Additional control studies were performed on blood exhibiting reticulocytosis of 3%–9% and obtained from subjects with uncomplicated, purely hemolytic syndromes. The range of reticulocytosis selected was dictated by its approximate comparability to that present in cord erythrocytes. While etiology of the hemolytic syndromes varied, most were hereditary. Both adults and children are included. Red cell enzymatic activities in children past the first few months of life differ little if at all from adult values. Specifically excluded were subjects whose reticulocytosis had been initiated by hematin therapy. In such subjects, metabolic activities are means of a new population and older cells present before therapy. Such bimodality renders them unsuitable as controls. Detailed data on these normal and reticulocyte-rich control, as well as cord, bloods have been previously reported.48

MATERIALS AND METHODS

The following glycolytic and hexose monophosphate (HMP) shunt enzymes were assayed by previously reported methods.49-54 These, together with abbreviations employed in text, figures and tables, included the following: hexokinase (HK), glucose-phosphate isomerase (GPI), phosphofructokinase (PFK), aldolase (ALD), triosephosphate isomerase (TPI), glyceraldehyde-3-phosphate dehydrogenase (G-3-PD), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), phosphopyruvate hydratase (enolase, ENOL), pyruvate kinase (PK), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PD), and 6-phosphogluconate dehydrogenase (6-PGD). The activity of the distal
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pentose shunt (DPS, ribulosephosphate-3-epimerase, phosphoriboisomerase, transketolase, and transaldolase) was screened in terms of production of fructose-6-phosphate (F-6-P) from ribose-5-phosphate (R-5-P). In addition, the following non-glycolytic enzymes were assayed by the referenced methodologies: lactoyl-glutathione lyase (glyoxalase I, GLY I) and hydroxacylglutathione hydrolase (glyoxalase II, GLY II), ribosephosphate pyrophosphokinase (RPK, PRPP synthetase) and adenine-phosphoribosyl transferase (APRT, glutathione peroxidase (GSH-Px), adenylate kinase (AK), and acetylcholine esterase (ACHE). Red cell glutathione (GSH) was measured by the method of Beutler et al. The following additional abbreviations have been employed: GSSG-R, glutathione reductase; Hb, hemoglobin; PCV, packed cell volume in ml/100 ml of blood; MCV, mean corpuscular volume in cu μ; SD, standard deviation.

RESULTS

General

The findings are summarized under case reports and in Table 1. Refractory anemia was usually moderate to severe, and reticulocytosis was most often absent or inappropriately low. Cases 14, 15, and 28 are exceptions to the latter and will be discussed in this regard. Leukopenia and thrombocytopenia were common but not invariable. Transferrin was more than 45% saturated in 16 of 21 subjects and was more than 70% saturated in ten of these. Other ferrokinetic abnormalities were noted in certain subjects. In five of eight cases, cytogenetic data were normal. In case 12 (Fanconi's anemia), the expected chromatid abnormalities were present, while in cases 13 and 16 a prominent cell clone with an extra "C" group chromosome was noted. In 11 cases, Hb F concentration was 2% or less, in two cases it was 4%-5%, and in one instance it varied from 2% to 4%.

Metabolic Studies

In Figs. 1–4, comparison of enzymatic activities and GSH content of erythrocytes in dyserythropoietic states is made with normal, reticulocyte-rich (3%–8.9%), and cord blood. For ease of comparison, activities are grouped in terms of those where cord blood is characteristically higher (Fig. 1), lower (Fig. 4), or roughly comparable to the controls with comparable reticulocy-
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<th>Group C</th>
<th>18 (79)</th>
<th>19 (46)</th>
<th>20 (81)</th>
<th>21 (64)</th>
<th>22 (73)</th>
<th>23* (70)</th>
<th>24 (62)</th>
<th>25 (58)</th>
<th>26 (73)</th>
<th>27 (55)</th>
<th>Group D</th>
<th>28 (51)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29</td>
<td>112</td>
<td>2.1</td>
<td>1.2</td>
<td>112</td>
<td>21</td>
<td>30</td>
<td>30</td>
<td>38</td>
<td>26</td>
<td>20</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>3.8</td>
<td>2.0</td>
<td>7.0</td>
<td>9.0</td>
<td>120</td>
<td>120</td>
<td>107</td>
<td>117</td>
<td>108</td>
<td>23.5</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Slight increase</td>
<td>Reduced</td>
<td>Low normal</td>
<td>Normal</td>
<td>Increased</td>
<td>Reduced</td>
<td>Normal</td>
<td>Reduced</td>
<td>Normal</td>
<td>—</td>
<td>Normal</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>—</td>
<td>54</td>
<td>96</td>
<td>81</td>
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<td>95</td>
<td>92</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>0.87†</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.04</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td></td>
<td>15</td>
<td>—</td>
<td>14-15</td>
<td>—</td>
<td>0.9</td>
<td>2.0</td>
<td>26.5</td>
<td>—</td>
<td>25</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Figures in parentheses are patient's age at the time of study. PCV, MCV, WBC, and reticulocyte count were those at time of study. T.I.B.C., total serum iron-binding capacity; P.I.T., plasma iron turnover (normal = 0.6 ± 0.2 mg iron/100 ml blood/day); $^{51}$Cr t 1/2 = labeled red cell half-time survival in days (normal = 26-32 days). Ferrokinetic data, $^{51}$Cr survival data, and Hb F values were obtained from most recent studies available.

*Some transfused cells present.
†During good response to corticosteroids.
Comparison of activities whose mean in cord blood is within 1 SD of mean of comparably reticulocyte-rich controls.

Omitted but briefly discussed later are similar data relative to HK. The latter activity is so greatly increased in young cells that graphic representation is difficult. It is obviously important to differentiate clearly those metabolic properties of red cells in both dyserythropoietic states and in cord blood that are unique and those that are possibly secondary to young mean cell age only. For each enzymatic activity expressed as a percentage of the normal control mean in Figs. 1-4, there is shown on the left the mean and SD for reticulocyte-rich controls and on the right a similar representation for cord blood. "Preleukemic" and frankly leukemic subjects and the single case with folate deficiency will be discussed separately.

Comparison with normal and reticulocyte-rich blood. Many activities (Figs. 1-4) are far higher than in either normal or high reticulocyte controls. Strictly comparable data for a control group with 3%-8.9% reticulocytosis are deemed inadequate for presentation of a mean ± SD for RPK, APRT, and ACHE. These are included in Fig. 4, however, since in our experience all have substantially elevated values in young cells and ACHE activity has even been widely employed as an index of red cell age, and cord blood erythrocytes are uniquely low in these activities.
METABOLIC FEATURES OF ERYTHROCYTES

This is true for all of the moieties shown in Fig. 1 where, for example, only one subject has GP1 or G-3-PD activity below the mean of the reticulocyte-rich controls. About 75% exceed the latter by more than 1 SD, and more than 35% exceed by greater than 2 SDs. PGK, AK, and GSH values in our laboratory differ little from normal in the presence of reticulocytosis. However, the majority of values for PGK and GSH reported here exceed the mean of both normal or high reticulocyte controls by more than 1 SD. In marked contrast, AK values in approximately 75% of cases are below the normal mean, and in more than 25% of cases are below by more than 1 SD. Approximately 45% of ENOL and 40% of G-6-PD values are more than 1 SD above the high reticulocyte control mean. Both very high, as well as very low, GSH-Px and PFK activities characterize certain cases. In these subjects, many activities are among the highest ever observed in this laboratory irrespective of the degree of reticulocytosis present.

PK activity deserves special mention. It is characteristically increased in young cells. In more than 75% of cases under study, it is, however, below the mean of high reticulocyte controls, and in more than 50% this difference exceeds 1 SD. In about 40% of cases, values are even lower than the normal mean.

Comparison with cord erythrocytes. Cord erythrocytes possess certain unique features. The mean activities shown in Fig. 1 exceeded the mean of comparably reticulocyte-rich controls by more than 1 SD. Those in Fig. 4 were decreased by more than 1 SD, while those in Figs. 2 and 3 have means within 1 SD of the mean of high reticulocyte blood. The latter are presumably not unique but deviate from normal approximately as expected for the degree of reticulocytosis present.

The erythrocytes in dyserythropoietic disorders frequently shared with cord blood the high activity pattern depicted in Fig. 1. A number of activities also coincided with the low values seen for PFK, GSH-Px, AK, and ACHE (Fig. 4). There are, however, striking dissimilarities as well.

(1) Many values exceed cord blood activity by more than 1 SD and some by several SDs, even when reticulocytosis is minimal.

(2) While the characteristically high activities of cord erythrocytes depicted in Fig. 1 are simulated in many instances, a substantial number of activities are more comparable to normal or reticulocyte-rich blood. The cord blood pattern is by no means uniformly evident.

(3) The characteristically low PFK activity of cord erythrocytes is simulated in less than 25% of cases and elevated PFK activity is more frequently noted.

(4) The low GSH-Px activity of cord erythrocytes is mimicked only rarely and, in contrast, many extremely high values exceed those of both cord and high reticulocyte controls, sometimes by several SDs.

(5) The relatively low RPK and APRT values of cord blood are most often not evident.

(6) Very importantly, PK values in cord erythrocytes are elevated as expected for the young mean cell age of cord blood. In 23 of 28 cases reported here, PK activity was below the mean of cord red cells. In 17 instances it was
decreased by more than 1 SD. In 15 cases, PK activity was also more than 1 SD below the mean of high reticulocyte controls. PK activity in the majority of the cases, then, did not conform to that of either young cells or cord blood. This was not invariable, and PK values are normal or appropriately increased in some subjects.

*Probably “preleukemic” or frankly leukemic subjects.* These deserve special mention because of their more diverse and bizarre hematologic features and the chromosomal abnormalities evident in cases 13 and 16. In case 15, the very high reticulocyte and nucleated erythrocyte count preclude valid comparison with any of the controls on a quantitative basis. To a lesser extent, the presence of 9% reticulocytes and a modest number of nucleated erythrocytes in the blood of case 14 might also raise some doubt as to the validity of comparisons. However, the activities observed clearly cannot be attributed simply to a youthful population. Case 15 has the highest HK activity we have ever observed (17-fold the normal mean), yet PK activity is less than half normal. If the normal mean HK/PK ratio is taken as 1, the ratio for case 15 is 40. Eight activities exceed the normal mean by threefold or more, yet TPI activity is normal, and ACHE activity is but 56% of normal. In case 14, while seven activities are two to six times greater than the normal mean, PK is but 32% and AK is 57% of normal. In case 13, six activities are threefold or more above the normal mean. ENOL activity is the highest we have observed (640% of the normal mean). In contrast, the activities of PK, PGK, AK, ACHE, and DPS are 81%, 104%, 37%, 86%, and 107%, respectively. Profound aberrations in enzyme ratios characterize these bizarre cases. Case 26 likewise has PK activity but 56% of normal, while HK, PGM, ENOL, and DPS are increased twofold or more. Case 27 has acute leukemia in remission but has been under continuous maintenance therapy with cytosine arabinoside and 6-thioguanine, agents known to affect nucleic acid metabolism. Despite his normal hemogram (except for some macrocytosis), he has very high values for HK, PFK, G-3-PD, PGK, ENOL, LDH, GSH-Px, GLY I, and APRT. PK activity is normal. Enzyme ratios are greatly disturbed, whether due to therapy (as seems most likely) or to underlying disease.

*Folate deficiency.* The single case of severe folate deficiency must be evaluated in terms of both the large reticulocyte population (23%) appearing in response to therapy and the pretherapy population derived during folate deprivation. The new cell population cannot serve as a full explanation for all of the abnormalities observed. For example, PGK and ENOL activities are several SDs above those expected even with the high degree of reticulocytosis present. In fact, PGK activity, which is usually increased very little as a result of reticulocytosis, is with a single exception the highest we have ever observed. While high PGK is also present in cord red cells, the greatly increased activity of GSH-Px and AK noted in case 28 clearly is the reverse of the low fetal pattern. AK in particular exhibited extremely high activity in a manner not characteristic of reticulocytosis or cord blood. Significantly, PK activity was increased as expected commensurate with the degree of reticulocytosis. The very high activity of ENOL in folate and B12 deficiency have been commented on in a previous publication.68
Enzyme ratios. Enzyme ratios must be interpreted with some caution and in the light of other data. Normal genetic variation is considerable. Moreover, a "normal" ratio can be achieved when activities compared are both high and low. Nonetheless, if over-interpretation is avoided, trends and gross differences can be readily appreciated. Table 2 compares cord, comparably reticulocyte-rich, normal, and case material erythrocytes in terms of three enzyme ratios. The ratios of the mean values of cord erythrocytes is arbitrarily defined as 1.0.

GSH-Px is uniquely decreased in cord blood, and GSH-Px/PK is on the average 2.5 times greater in comparably reticulocyte-rich blood. In the cases reported here, this ratio is as low as that of cord blood in but one case. In 18 cases, ratios exceed those of cord blood by 3.5 times and in six cases by sixfold or more. In 16 cases, the mean ratio of reticulocyte-rich controls is also exceeded by 50% or more. Cases 14 and 15 have exceedingly high ratios.

PGK is uniquely high, and PFK is characteristically somewhat low in cord erythrocytes. The ratio PGK/PFK in the 28 cases under study was lower than the cord blood mean in 24 cases. In 20 cases, it is 75% or less and in nine cases it is 50% or less. Most ratios are comparable to those of normal or high reticulocyte control blood.

The HK/PK ratio is virtually the same in cord and comparably reticulocyte-rich red cells. PK in many instances was inappropriately low in the erythrocytes of the case material presented here. Seventeen ratios exceed those of both cord and high reticulocyte blood by 50% or more, 11 ratios exceed by more than 100%, and five ratios exceed by 200% or more. The ratios of cases 14 and 15 are exceedingly high. It is clear that not only absolute enzyme activities, but also erythrocyte enzyme ratios in dyserythropoietic states often differ greatly from those of normal, cord, or simply "young" red cells.

DISCUSSION

The foregoing data document marked aberrations in metabolic activities in a diversity of dyserythropoietic disorders. Some resemble the patterns of cord erythrocytes, but there are also striking differences, as previously discussed. Though the refractory anemias are very heterogeneous, strong threads of similarity in metabolic patterns often are observed from case to case. These similarities, as well as similarities to cord blood, might be explained in terms of partial reversion to a fetal or embryonal form of erythropoiesis.31 While such a unifying hypothesis is attractive and is compatible with some of the findings, it fails to explain others. It seems inherently unlikely in such a diverse group of both hereditary and acquired disorders that repression and derepression of genes in the pattern of the fetus is a sole common denominator. What other explanation could be invoked, then, for the frequently striking similarity of findings from case to case and at the same time afford some insight into the differences and inconsistencies?

In a broad sense, the dyserythropoietic disorders have in common karyoand cytokinetic abnormalities and evidence of disturbed nucleic acid metabolism. These are morphologically suggested by erythroid multinucularity, the
Table 2. Enzyme Ratios in Dyserythropoietic Disorders*

<table>
<thead>
<tr>
<th>Case</th>
<th>Group A GSH-Px/PGK/ HK/ PK</th>
<th>Group B GSH-Px/PGK/ HK/ PK</th>
<th>Group C GSH-Px/PGK/ HK/ PK</th>
<th>Group D GSH-Px/PGK/ HK/ PK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH-Px/ PGK/ HK/ PK</td>
<td>GSH-Px/ PGK/ HK/ PK</td>
<td>GSH-Px/ PGK/ HK/ PK</td>
<td>GSH-Px/ PGK/ HK/ PK</td>
</tr>
<tr>
<td>1</td>
<td>4.1/0.5/1.4</td>
<td>13/3.6/0.4</td>
<td>19/5.2/0.6</td>
<td>28/2.0/0.6/1.2</td>
</tr>
<tr>
<td>2</td>
<td>2.6/0.6/1.0</td>
<td>14/24.5/0.4</td>
<td>20/0.9/0.6</td>
<td>Normal controls</td>
</tr>
<tr>
<td>3</td>
<td>2.7/0.7/1.6</td>
<td>15/27.4/0.4</td>
<td>21/4.3/1.2</td>
<td>2.7/0.6/0.7</td>
</tr>
<tr>
<td>4</td>
<td>2.7/0.6/1.1</td>
<td>16/---/0.6</td>
<td>22/4.7/0.5</td>
<td>High reticulocyte controls</td>
</tr>
<tr>
<td>5</td>
<td>2.2/0.7/1.1</td>
<td>17/4.6/0.5</td>
<td>23/3.8/0.7</td>
<td>(3%&lt;8.9%)</td>
</tr>
<tr>
<td>6</td>
<td>7.4/0.5/1.8</td>
<td>18/3.5/0.8</td>
<td>24/5.1/0.6</td>
<td>2.4/0.5/0.9</td>
</tr>
<tr>
<td>7</td>
<td>6.4/0.9/2.2</td>
<td>25/2.7/0.5</td>
<td>27/8.1/0.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.0/0.8/2.1</td>
<td>26/8.3/0.6</td>
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<tr>
<td>9</td>
<td>4.6/1.0/1.6</td>
<td>27/8.1/0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.3/0.5/1.1</td>
<td></td>
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</tr>
<tr>
<td>12</td>
<td>3.8/1.1/2.0</td>
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</table>

*Ratio of mean values of cord blood arbitrarily defined as 1.0.
Megaloblastosis of folate deficiency, the frequency with which megaloblastoid marrow features are described, the peripheral macrocytosis and sometimes "partial hypochromia," the prominent marrow clones with extra chromosomes in two cases, the chromatid breaks and gaps in the one case of Fanconi's anemia, and the high incidence of intramedullary cell death characterizing all forms of ineffective erythropoiesis in general. In the presence of disturbed cell maturation and division, several possible explanations of inordinately high enzyme activities are tenable. Failure of cell division could prevent the distribution of already synthesized enzyme proteins to daughter cells. Failure of maturation might lead to a longer total period of enzyme synthesis prior to nuclear pyknosis and loss of chromosomal functions. Finally, increase in average DNA content, extra chromosomes, multinucleated cells, and endoreduplication could on a gene-dose basis enhance the amount of enzyme proteins produced per cell. A varied pattern of repressed and derepressed genes resembling that of the fetus would likewise not be excluded.

However, delays in maturation or cell division do not, per se, offer an explanation for more than elevated activities. They do not explain gross aberrations in enzyme ratios, low activities, or sporadically elevated activities of a given enzyme in apparently capricious fashion. Moreover, metabolic patterns characterizing the very heterogeneous disorders reported here are by no means entirely haphazard or random. Rather, there are recurrent themes that frequently permit the correct prediction of dyserythropoiesis without reference to clinical data. Interestingly, however, these do not differentiate between a diversity of hereditary and acquired dyserythropoietic states. In precursor cells of the uniquely anucleate human erythrocyte, protein synthesis normally abates and finally ceases as nuclear chromatin degenerates and cytoplasmic organelles are lost. It is not known whether synthesis of various enzymic cell constituents subsides simultaneously, randomly, or in accord with some roughly ordered asynchronism. If the latter should pertain, metabolic similarities between syndromes with different etiologies might represent partial adherence (albeit with exaggerations and distortions) to phenomena normally under regulation. Speculatively, despite the multiplicity of possible cyto- and karyokinetic abnormalities, the dyserythropoietic disorders in general might retain some semblances to (as well as departures from) a normally ordered and asynchronous loss of genetic material governing enzyme synthesis. In these circumstances, aberrations in enzyme activities residual in a cell incapable of further protein synthesis might be undifferentiable from those alternatively explainable in terms of genic repression or derepression.

That such alterations in chromosomal function and in the pattern of nuclear maturation and degeneration actually occur is supported by certain data. For example, in CDA Type I (cases 1 and 2), light and electron microscopy studies of erythroblasts have revealed characteristic morphologic aberrations with widening of nuclear membrane pores, condensation, vacuolation and disintegration of nuclear chromatin, structural changes of the nucleolus, and finally autolysis of the cells. In cells with two nuclei, the latter vary in size, in degree of degeneration, and in staining properties. Meuret et al., in extensive studies of DNA, RNA, histone, and Hb content in CDA I, have
found the DNA content of a high proportion of erythroblasts to be hyper-tetraploid. They concluded that DNA synthesis was not coordinated with the normal cell cycle and that this was morphologically manifested by enlarged nuclei, incomplete nuclear division, chromatin bridges between nuclei, and multinuclearity. Altered deoxyribonucleoprotein metabolism was present in the early erythroblasts, and RNA synthesis was also markedly reduced causing a disturbance in Hb synthesis. In B12 and folate deficiency chromosome studies, radioautography and DNA measurements in individual cells have shown extensive chromosomal abnormalities and a pattern of DNA distribution entirely unlike normal counterparts.\textsuperscript{20,69} Evidence has been presented for disturbances in both the mitotic and intermitotic phases of the cell cycle.\textsuperscript{20,31} Interestingly, the chromosome changes have appeared to be isolated events in individual cells without evidence of stable cell lines or clones. Such a pattern, if applicable to other dyserythropoietic states, would be compatible with unpredictable metabolic variations from case to case. Agents known to interfere with DNA metabolism, such as cytosine arabinoside, have likewise been shown to produce similar chromosomal changes.\textsuperscript{70,71} Case 17, for example, had been on long-term maintenance therapy with this agent. Morphologic alterations of erythroid precursors in dyserythropoietic disorders have been extensively illustrated and discussed by Lewis.\textsuperscript{72-74} While sophisticated studies are unavailable on the entire spectrum of dyserythropoietic states, light microscopy itself often reveals suggestive changes in nuclear morphology compatible with disturbances in DNA metabolism and nuclear chromatin.

Some mention should be made of cases 13 and 16, both of which had an extra “C”-group chromosome present in an apparently stable and prominent clone of cells. In case 13, the striking elevations in certain enzyme activities tempted the thought that these might result from the presence on the extra chromosome of the gene governing their production. For example, ENOL activity in case 13 was some 15 SDs above the mean of cord blood and was considerably more above that of reticulocyte-rich controls. Such a simplistic explanation is rendered unlikely, however, by the very magnitude of the increased activities and by the fact that X-chromosome-linked G-6-PD also was greatly increased in activity.

Cases 6–9, while not proved identical, are strikingly similar clinically, morphologically, and metabolically. Cases 6 and 7 are siblings, so that their presumably hereditary nature appears virtually assured. Cases 7 and 8 are considered probably congenital, but the absence of affected siblings and the fact that they were not specifically detected in the neonatal period precludes dogmatism on this point. Three of the four cases have red cell PK activity comparable to or below the normal mean, and the fourth also has relatively low PK activity. These cases, we believe, may well represent a distinct type of congenital dyserythropoiesis, but final proof is still lacking. They most closely resemble a syndrome described by Schröter as “Chronische idiopathische infantile Panzytopenia” with relative PK and GSSG-R deficiency.\textsuperscript{75} His data indicate unusual elevations in many enzymes as well. Schröter believed his cases were nonfamilial, chiefly because of lack of affected siblings and normal findings in parents. It should be recalled that in our cases, even with sibling
involvement, no metabolic or hematologic abnormalities could be found in parents. Schrötter's cases differed from ours in having somewhat greater values for Hb F, but the significance of this as a truly differential point is doubtful.

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Dyserythropoiesis, Refractory Anemia, and "Preleukemia:" Metabolic Features of the Erythrocytes

William N. Valentine, Patricia N. Konrad and Donald E. Paglia