Recessive Familial Erythrocytosis: Aspects of Marrow Regulation in Two Families

By John W. Adamson, George Stamatoyannopoulos, Stella Kontras, Andre Lascari, and James Detter

Studies of marrow regulation were carried out in two families with recessively expressed erythrocytosis. The erythrocytosis in the affected individuals was associated with increased erythropoietin (ESF) production. However, hormone production was uninfluenced by alterations in the O₂ carrying capacity of the blood when the hematocrit was lowered acutely by phlebotomy. Hemoglobin and red cell function (p50; 2, 3-diphosphoglycerate) in the affected, as well as the unaffected, family members were normal. Renal arteriography revealed no mass lesions and no anatomic abnormalities of the renal macro- or microvasculature. Antisera capable of neutralizing the biological activity of normal human ESF completely inhibited the erythropoietic activity in the serum and urine of those studied. Since increased production of ESF occurred in the absence of recognizable physiologic requirements and since the normal reciprocal relationship between O₂ delivery and hormone production was absent, the most likely explanation for this clinical constellation is a cellular defect in the regulation of production of ESF or of its precursor substances.

Numerous reports of erythrocytosis or polycythemia appearing in multiple members of a single family have appeared over the years and recently have been reviewed. Various designations have been used to describe these clinical syndromes, including “benign familial erythrocytosis” or “polycythemia vera of childhood,” names that stressed the nonprogressive course of the disease, its familial character, and the fact that secondary causes of erythrocytosis were not evident. The underlying pathophysiology of these disorders, however, remained unclear until 1966 when Charache et al. described Hb Chesapeake, a mutant hemoglobin characterized by increased O₂ affinity and associated with erythrocytosis in affected family members. Since increased production of ESF occurred in the absence of recognizable physiologic requirements and since the normal reciprocal relationship between O₂ delivery and hormone production was absent, the most likely explanation for this clinical constellation is a cellular defect in the regulation of production of ESF or of its precursor substances.
members. This finding led to the subsequent discovery of a number of mutant hemoglobins with similar physiologic properties and provided a rational physiologic and biochemical approach to the evaluation of the familial erythrocytoses. In addition, studies in individuals with hemoglobins of high \( O_2 \) affinity have led to a better understanding of the relationship between \( O_2 \) transport mechanisms and marrow regulation.

Genetic analysis of all the published families with erythrocytosis indicates that, in addition to the dominant expression of the abnormally functioning mutant hemoglobins, there are families in which erythrocytosis appears as a recessive defect. This contention is supported by the fact that the propositi arise from hematologically normal parents, that the corrected segregation ratio of affected:nonaffected in acceptable cases is 1:3, and that consanguinity has been clearly documented in at least one family. The pathogenesis of increased red cell production in these forms of familial erythrocytosis is unknown.

This report presents a systematic investigation into aspects of marrow regulation in two such families. The studies were designed to define the \( O_2 \) transport characteristics of red cells and hemoglobin in these individuals and to determine the interdependence of erythropoietin (ESF) and \( O_2 \) delivery in marrow regulation.

**CASE REPORTS**

**Family E**

The propositus (II-1; Fig. 1) was the product of a normal pregnancy, although delivery was induced at 39 wk. Hyperbilirubinemia was marked in the immediate postnatal period, but the child did well and exchange transfusion was not required. At age 2½ yr he was evaluated for "failure to thrive," at which time an elevated hemoglobin (17.1 g) was documented. Two months later, he was evaluated at the University of Iowa Hospitals for polycythemia. At that time the hemoglobin was 17.7 g/100 ml, hematocrit 67, WBC 9100/cu mm with a normal differential, and platelet count 180,000/cu mm. Except for his small size, the physical examination and vital signs were unremarkable. Work-up included normal blood gases (P\( O_2 \) 107 mm Hg, P\( CO_2 \) 32.5 mm Hg, PH 7.38, \( O_2 \) saturation 98%), normal chest x-ray, intravenous pyelogram (IVP), and electrocardiogram. Bone marrow examination revealed only mild erythroid hyperplasia. The child did reasonably well until 2 yr later when, several hours following apparently mild head trauma, he began vomiting, became progressively more lethargic, obtunded, and died. No autopsy was performed.

The brother (II-2) of the propositus, studied at 18 mo of age, is now a healthy, well-developed 3-yr-old. He was the product of an uneventful pregnancy and delivery. Hematocrit and hemoglobin at birth were 77 and 24.6 g/100 ml, respectively; otherwise he was well. At age 7 mo he was evaluated for polycythemia at the University of Iowa Hospitals, at which time his hematocrit and hemoglobin were 57 and 18.4 g/100 ml, respectively, and he was iron deficient (serum iron 35 \( \mu \)g/100 ml, total iron-binding capacity 609 \( \mu \)g/100 ml, 6% saturation). WBC and platelet counts were normal. The remainder of the evaluation, including chest x-ray, IVP, brain scan, skull x-rays, repeated urinalyses, blood chemistries, and blood gases (P\( O_2 \) 88 mm Hg, P\( CO_2 \) 38 mm Hg, PH 7.39, \( O_2 \) saturation 96%), was normal. Subsequent studies, including renal arteriography and measurement of plasma renin levels by both bio- and immunoassay, were normal (patient, 95 ng/100 ml; normal range, 100-400 ng/100 ml). The red cell mass was 65 cc/kg (hct. 72) using \( ^{51} \)Cr-labeled autologous red cells.
RECESSIVE FAMILIAL ERYTHROCYTOSIS

Fig. 1. Inheritance patterns of recessively expressed erythrocytosis in two families. Hematocrits shown represent maximum values obtained during initial work-up of these subjects.

There was no family history of polycythemia, leukemia, or other blood dyscrasias, and consanguinity could not be implicated by history.

Family F

This family, also without evident consanguinity, has been investigated previously.16 Subsequent to that report, the propositus (II-1; Fig. 1) and his unaffected brother (II-3) died in separate motor accidents. Autopsies were not performed. The surviving sister (II-2) has erythrocytosis but is otherwise in good health. Renal arteriography performed in II-2 revealed an accessory right renal artery that was considered a normal variant. No other abnormalities of the macro- or microvasculature were found. Plasma renin levels were low (70 ng/100 ml).

MATERIALS AND METHODS

The surviving affected members of both families and their available parents were admitted to the Clinical Research Center, University of Washington Hospital, Seattle, Wash.

Hematologic Studies

Routine hematologic values were obtained using standard techniques. Additional screening laboratory tests included an absolute basophil count and leukocyte alkaline phosphatase (LAP) score. Direct measurement of the red cell mass using 51Cr-labeled autologous red cells was performed in F(II-2).

Hemoglobin and Red Cell Function Studies

Hemoglobin studies, performed on fresh hemolysates, included electrophoresis on starch gel at alkaline pH (8.6) and on agar gel at near neutral pH (6.5), and chromatography on DEAE-Sephadex and carboxymethyl (CM) cellulose Sephadex using previously established techniques.12 Red cell O₂ affinity was examined in heparinized blood samples, and the O₂-hemoglobin dissociation curve, Bohr effect factor, and Hill's n were determined as described by Lenfant, et al.17 Oxygen dissociation characteristics were also measured in dialyzed hemoglobin solutions. Red cell 2,3-diphosphoglycerate (DPG) and ATP levels were determined by the method of Bartlett.18
Erythropoietin Studies

Urine for ESF determination was collected and processed as previously described. Specimens were collected for a minimum of 3 days prior to and 5 days following a single isovolemic phlebotomy designed to reduce the red cell mass by at least 15%. The phlebotomy was performed during the fourth day of study. Blood removed was replaced quantitatively with an appropriate plasma expander (Albumisol). A 25% aliquot of each pooled daily collection was concentrated to a final volume of 20 ml and assayed in ex-hypoxic polycythemic mice. There were six mice in each experimental group, and each animal received 1.0 ml of concentrate on 2 consecutive days. The pre- and post-phlebotomy means of radioiron incorporation of animals receiving test material were compared statistically and then converted to units ESF by comparison to a simultaneously determined ESF Standard B dose-response relationship. Erythropoietin neutralization studies were carried out using antisera developed in rabbits against urinary ESF from anemic human sources. The techniques of antibody development and the neutralization procedures followed those previously described by Schooley and Garcia. The quantity of antisera employed was calibrated from bioassay results to be that amount just capable of the predicted complete neutralization of the biological activity from the serum of subject E(II-2) and the urine of F(II-2). In addition, F(I-2) was hospitalized for similar physiologic studies as an obligatory heterozygote.

Studies of Erythropoiesis and Iron Metabolism

Serum iron and total iron-binding capacity were measured using recently recommended techniques. Iron kinetic studies, using the method of Finch et al., were performed before and then 5 days after phlebotomy in the mother and daughter of family F. Blood samples were obtained twice daily following the injection of radioiron, in order to characterize the rate of reappearance of the label in newly formed red cells as a determinant of the marrow transit time (MTT).

RESULTS

Hematologic Studies

The routine hematologic studies, summarized in Table 1, documented erythrocytosis in the children of both families. The measured $^{51}$Cr red cell mass in F(II-2) was 35.5 cc/kg (normal: 25–30 cc/kg). No consistent elevation in other cell counts was evident, and the basophil count and LAP scores were normal. The peripheral blood smears of both children were remarkable in that there was an increase in the number of macroreticulocytes ("shift cells"); in the case of E(II-2), occasional nucleated red cells (less than 1 per 100 WBC) were also found.

Hemoglobin and Red Cell Function Studies

The hemoglobin of all family members was electrophoretically and chromatographically normal. The results of the determinations of hemoglobin function are summarized in Table 2. Whole blood p50 values in both families failed to document abnormalities of the O2-hemoglobin dissociation curve similar to those seen with mutant hemoglobins. Only E(II-2) had a value other than normal, and this rightward displacement of the dissociation curve was accounted for by the increased level of red cell 2, 3-DPG. Dissociation curves obtained in dialyzed hemoglobin solutions did not differ from those of normal control subjects. Values for Hill's n were normal in each individual, indicating no discernible abnormality of heme-heme interaction.
Table 1. Hematology Values

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Hematocrit</th>
<th>Hb</th>
<th>Reticulocyte Count*</th>
<th>MCV</th>
<th>Red Cell Indices</th>
<th>MCH</th>
<th>MCHC</th>
<th>Platelet Count</th>
<th>WBC</th>
<th>SeFet/TIBC</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>I-1</td>
<td>33</td>
<td>44.0</td>
<td>15.9</td>
<td>0.9</td>
<td>87</td>
<td>31.0</td>
<td>36.0</td>
<td>258,000</td>
<td>7,400</td>
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<tr>
<td>I-2</td>
<td>32</td>
<td>36.8</td>
<td>13.5</td>
<td>1.1</td>
<td>89</td>
<td>32.0</td>
<td>36.6</td>
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<tr>
<td>II-2</td>
<td>1.5</td>
<td>66.4</td>
<td>17.2</td>
<td>2.2</td>
<td>64</td>
<td>17.4</td>
<td>26.6</td>
<td>160,000</td>
<td>9,900</td>
<td></td>
<td>23/450</td>
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<tr>
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<tr>
<td>I-2</td>
<td>47</td>
<td>40.0</td>
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<td>0.8</td>
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<td>1.8</td>
<td>76</td>
<td>23.0</td>
<td>30.3</td>
<td>308,000</td>
<td>8,300</td>
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*Not corrected.
†Serum iron as μg/100 ml plasma.

Table 2. Characteristics of Hemoglobin Function

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hct (%)</th>
<th>p 50*</th>
<th>Bohr Effect Factor</th>
<th>Hill's n +</th>
<th>2,3-DPG μmol/g Hb</th>
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<tr>
<td>I-1</td>
<td>44.0</td>
<td>25.0</td>
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<tr>
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<td>36.8</td>
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<td>-0.550</td>
<td>2.25</td>
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<tr>
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<td>66.0</td>
<td>31.1</td>
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<td>2.35</td>
<td>6.50</td>
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<td></td>
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<td></td>
</tr>
<tr>
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<td>26.0</td>
<td>-0.440</td>
<td>2.55</td>
<td>4.91</td>
</tr>
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</table>

*As mm Hg. Normal value, 26.8 (SD ± 0.6) for whole blood.
†Hill's equation is: \( \log \left( \frac{Y}{1-Y} \right) = n \log P_{O_2} + \log K \), where \( Y \) is the degree of oxygenation, \( P_{O_2} \) is oxygen pressure, and \( n \) and \( K \) are constants.
‡As μmoles/cc packed red cells. Normal range, males and females: 4.8 (SD ± 0.3) and 5.3 (SD ± 0.3) and 5.3 (SD ± 0.4), respectively.
Erythropoietin Studies

Daily ESF excretion pre- and postphlebotomy in the subjects with erythrocytosis is illustrated in Fig. 2. Daily excretion in E(II-2) averaged 4.1 Standard B units/day and ranged from 2.5 to 7.0 U through the day of phlebotomy. Previous attempts to quantitate ESF excretion in the urine of children of this age have been generally unsuccessful (Adamson, J. W., unpublished observations). For F(II-2), ESF excretion ranged from 4.8 to 8.1 U/day and averaged 6.3 U, at the upper limits of the normal range for adults studied by these methods but higher than the few patients of this age previously investigated. (Adamson, J. W., unpublished observations). Significantly, no increase in ESF excretion was seen in either subject in response to the phlebotomy. Serving as a heterozygote control, F(I-2) behaved normally (Fig. 3). Four of 5 postphlebotomy study days gave statistically significantly (p < 0.05) greater values of $^{59}$Fe incorporation than any prebleed day, and the pattern of increase was similar to those previously reported. Basal ESF excretion of approximately 1 U/day rose by 230% when the hematocrit was lowered acutely.

Erythropoietin excretion before and after phlebotomy in our patients was compared to normal volunteers and to individuals with erythrocytosis associated with mutant hemoglobins by plotting the change in ESF excretion...
Fig. 3. Daily ESF excretion and hematocrit before and after acute phlebotomy in the obligatory heterozygote, F(I-2).

(as Δ log ratio) against the per cent reduction in hematocrit. As shown in Fig. 4, there is a clear difference between normal subjects and those with familial erythrocytosis of different etiologies. The heterozygote, F(I-2), had a normal pattern of response when compared to control subjects.

Erythropoietin was easily measurable in the serum of E but not F. Studies performed with antisera capable of neutralizing normal human ESF are...
shown in Fig. 5. These studies document the virtual complete neutralization of assayable activity in specimens from these subjects.

**Studies of Iron Metabolism and Erythropoiesis**

Both children were iron deficient, and its significance was reflected by the reduction in red cell indices (Table 1). While iron deficiency precludes some aspects of quantitative kinetic studies, the MTT retains its usefulness as an indirect monitor of ESF activity. Thus, commensurate with the level of increased ESF stimulus in F(II-2), the MTT was shortened to 2.5 days and did not change significantly with phlebotomy (2.4 days), as would have been expected even in the presence of iron deficiency had ESF production significantly increased. In F(I-2), on the other hand, the MTT was 3.9 days at a normal hematocrit. Following phlebotomy, the MTT shortened appropriately to 2.5 days, in spite of the fact that the phlebotomy induced a state of iron deficiency in this subject as well. Unfortunately, iron deficiency also invalidated the reticulocyte response to bleeding in all of the subjects.

**DISCUSSION**

The regulation of erythropoiesis in normal man is complex, balanced on those factors contributing to ESF production, O₂ delivery and marrow function. Mutations conceivably could be expressed at a number of steps in the regulatory chain, however the molecular mutant hemoglobins are the only distinct abnormalities known presently that result in erythrocytosis. Although techniques are lacking for the complete evaluation of a number of the regulatory steps, one may generally approach the clinical problem of polycythemia through a combination of studies of hemoglobin function and ESF production.

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**Fig. 5.** Effect of rabbit antihuman ESF serum on erythropoietic activity of serum and urine obtained from subjects with recessive familial erythrocytosis.
In man, erythrocytosis may be physiologically divided into three categories: tissue hypoxia; autonomous ESF production, as from neoplastic sites; and autonomous marrow production, as exemplified by polycythemia vera. Basal ESF excretion in patients with tissue hypoxia is at least normal and usually variably elevated, while in polycythemia vera or in normal individuals made polycythemic by autohypertransfusion, ESF excretion is subnormal or undetectable. When subjected to phlebotomy, however, both patients with hypoxia-induced erythrocytosis and those with polycythemia vera are able to increase measurable urinary ESF appropriately. In tumor-associated erythrocytosis, on the other hand, ESF excretion, while normal or increased in the basal state, appears independent of the O₂ content of the blood. Thus, ESF production and other parameters of erythropoiesis remain unchanged over a wide range in hematocrit. These characteristics of the response to phlebotomy provide insight into the nature of marrow regulation in certain pathologic conditions.

With this approach, the clear relationship between O₂ availability and erythropoiesis was documented for individuals with erythrocytosis associated with mutant hemoglobins Yakima and Rainier. In these settings, the O₂-hemoglobin dissociation curve is shifted leftward, denoting an increased affinity of the hemoglobin molecule for oxygen. This results in hypoxia at the tissue level and an increase in ESF production. Compensation for tissue hypoxia is achieved by a modest increase in the red cell mass without attendant increases in red cell 2,3-DPG or cardiac output. The subjects reported here have no discernible abnormalities of O₂ transport function by red cell or hemoglobin. In spite of the documented erythrocytosis, O₂ dissociation curves in whole blood and hemoglobin solutions were normal or shifted rightward E(II-2), and in this instance the increased p50 could be accounted for by the increase in red cell 2,3-DPG. While the mechanism underlying the observed increase in 2,3-DPG is unclear, a number of factors, including a reduced cardiac output, may result in an increased p50 without producing erythrocytosis. In any event, these findings exclude the possibilities that red cell overproduction is associated with a mutant hemoglobin or with defects in enzyme systems capable of altering the availability of 2,3-DPG. Insight into the nature of the alteration in erythropoietic regulation was provided by physiologic studies of ESF production over a wide range in O₂ availability. The persistent production of ESF at an elevated hematocrit disqualifies these individuals as having a familial form of polycythemia vera. In addition, the erythrocytosis does not appear to be regulated by tissue O₂ requirements. Thus, when the O₂ carrying capacity of the blood was reduced by phlebotomy, there was no demonstrable increased in ESF excretion. Consequently, the predicted shortening in MTT did not occur. Previous studies have demonstrated a direct relationship between hematocrit and MTT in normal individuals subjected to phlebotomy and in patients with hemolytic disease of various kinds. More recently, studies in patients with simple iron deficiency have demonstrated that this general relationship holds. Thus, it would be expected that an MTT of 2.5 days would be reduced by about 1.0 day in response to a 10-point
The pattern of hormone production in these patients is similar to that described previously for patients with tumor-related erythrocytosis. The insensitivity to phlebotomy, coupled with normal renal arteriography and normal plasma renin levels, would seem to rule out an abnormality of the renal microvasculature similar to that recently reported.29

The precise abnormality of ESF regulation in our patients is unclear, but there are several possibilities. First, it is possible that an undisclosed benign tumor or neoplasm giving rise to ESF or an ESF-like substance is present in these individuals. Careful neutralization studies indicate that the measurable erythropoietic activity is similar to, if not identical with, normal human ESF, thus making it unlikely that we are dealing with a molecular mutant of the hormone. Another possibility to be considered is that there is an abnormality of the renal sensor normally operative in regulating ESF production. This is not evident as a simple “resetting” of the O2 sensor (thus the nonresponsiveness to change in O2 carrying capacity) but more likely an abnormality at the intracellular site of ESF generation. In terms of the postulated renal enzyme/plasma substrate mechanism of Gordon et al.,30 the defect might lie in the production of either the plasma substrate or erythropoietin, the renal enzyme. These various possibilities cannot be tested at present.

It is possible that phlebotomy to a more significant degree of anemia might have provided information on the question of ESF arising by two different mechanisms or from two different sites. Thus, had the hematocrit been reduced to 30%, one might be able to measure an increment of additional ESF production presumably mediated by the normally functioning renal center.

Clinically, the affected members of families similar to the two reported here differ from those characterized by the mutant hemoglobins in that the hematocrit and hemoglobin levels of the former are distinctly higher.1 While the manifestations of the erythrocytosis appear to be mild, the long-term evaluation of a significant number of such individuals has not been possible. Iron deficiency undoubtedly has protected these patients by preventing an even more marked elevation in hematocrit and hemoglobin. This clinical constellation should not be considered “benign” in the sense that it requires no therapeutic intervention. The affected individuals in these families are subjectively improved by phlebotomies designed to maintain their hematocrit levels below 55%. One can only speculate on the relationship of erythrocytosis to the presumed intracranial hemorrhage that led to the death of the propositus in family E; however, in the absence of documented physiologic requirements for the increased red cell mass, it seems preferable to avoid the potentially adverse effects of chronic hyperviscosity in these individuals.

ADDENDUM

Since the preparation of this manuscript, subject II-2, Family E, was admitted to the University of Iowa Hospital with a stroke syndrome and hematocrit of 72%. It is the authors’ opinion that the hyperviscosity attending this clinical condition must be carefully controlled with phlebotomy to prevent the occurrence of vascular accidents such as this.
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