Impaired Erythropoietin Response in Anemic Premature Infants

By George R. Buchanan and Allen D. Schwartz

Serum erythropoietin (ESF) levels were measured by the polycythemic mouse bioassay in a group of normal premature infants with physiologic anemia. No ESF was detected in these infants, while a group of older anemic children with similar hematocrit values had elevated levels. An impaired ESF response in these infants may be an important factor in the pathogenesis of the anemia of prematurity. Tissue oxygen requirements in the premature infant are probably met in some way despite diminished ESF production and low hematocrit levels.

NORMAL FULL-TERM infants develop anemia during the second through the fourth months of life, during which time the nadir of the hematocrit ranges from 25% to 33%. This is often referred to as a state of physiologic anemia. In premature infants the anemia is more pronounced and begins earlier in life. This anemia of prematurity is found in seemingly completely normal infants, can be severe with hematocrits as low as 20%, and is unresponsive to hematinics such as iron or folic acid.

The exact etiology of physiologic anemia of full-term and premature infants is unknown. A mildly decreased red cell survival and the infant's extremely rapid growth with resultant increase in blood volume are certainly contributing factors, both of which are more pronounced in the premature than in the full-term infant. However, ferrokinetic and morphologic data indicate that decreased bone marrow erythroid activity is the major factor responsible for physiologic anemia. It is unknown whether or not this relative bone marrow erythroid failure occurs in the presence of adequate erythropoietin (erythropoiesis-stimulating factor or ESF) stimulation to the marrow. The present study was therefore undertaken to measure serum ESF levels in anemic premature infants.

MATERIALS AND METHODS

Patients

Blood specimens were obtained from 16 premature infants on the wards and in the nurseries of the Northwestern University Medical Center Hospitals and the premature nursery at Grant Hospital (Table 1). The infants were of varied birth weights (765-2325g, mean 1272) and gestational ages (27-35 wk, mean 32). They were of the postdelivery age at which physiologic anemia is most pronounced (31-86 days, mean 51). The infants were generally healthy at the time the blood samples were obtained, although many had had respiratory problems in the immediate newborn period. Two of the infants (V.K. and C.S.) had previously been transfused. All were...
Table 1. Data on the Premature Infants

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (days)</th>
<th>Birth Weight (g)</th>
<th>Hematocrit (%)</th>
<th>Hemoglobin (g/100 ml)</th>
<th>No. of Assay Animals</th>
<th>Mean 59Fe Incorporation (%) (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.F.</td>
<td>35</td>
<td>1389</td>
<td>19</td>
<td>6.5</td>
<td>3</td>
<td>0.33 (0.29-0.39)</td>
</tr>
<tr>
<td>L.G.</td>
<td>39</td>
<td>1049</td>
<td>23</td>
<td>7.5</td>
<td>3</td>
<td>0.44 (0.26-0.69)</td>
</tr>
<tr>
<td>M.A.</td>
<td>55</td>
<td>1100</td>
<td>23</td>
<td>7.7</td>
<td>4</td>
<td>0.46 (0.24-0.85)</td>
</tr>
<tr>
<td>V.K.</td>
<td>51</td>
<td>1162</td>
<td>24</td>
<td>8.6</td>
<td>4</td>
<td>0.40 (0.30-0.53)</td>
</tr>
<tr>
<td>T.S.</td>
<td>37</td>
<td>1276</td>
<td>24</td>
<td>8.5</td>
<td>3</td>
<td>0.59 (0.47-0.73)</td>
</tr>
<tr>
<td>C.S.</td>
<td>69</td>
<td>1180</td>
<td>24</td>
<td>8.5</td>
<td>3</td>
<td>0.30 (0.23-0.40)</td>
</tr>
<tr>
<td>E.B.</td>
<td>37</td>
<td>765</td>
<td>24.5</td>
<td>8.8</td>
<td>3</td>
<td>0.45 (0.37-0.53)</td>
</tr>
<tr>
<td>C.C.</td>
<td>86</td>
<td>2325</td>
<td>25</td>
<td>8.1</td>
<td>3</td>
<td>0.30 (0.24-0.38)</td>
</tr>
<tr>
<td>N.K.</td>
<td>50</td>
<td>1247</td>
<td>26</td>
<td>9.1</td>
<td>3</td>
<td>0.40 (0.33-0.52)</td>
</tr>
<tr>
<td>R.E.</td>
<td>62</td>
<td>1928</td>
<td>27</td>
<td>8.3</td>
<td>5</td>
<td>0.55 (0.33-0.88)</td>
</tr>
<tr>
<td>B.A.</td>
<td>56</td>
<td>1233</td>
<td>27</td>
<td>9.0</td>
<td>4</td>
<td>0.39 (0.24-0.49)</td>
</tr>
<tr>
<td>D.C.</td>
<td>31</td>
<td>1304</td>
<td>29</td>
<td>10.3</td>
<td>4</td>
<td>0.33 (0.26-0.39)</td>
</tr>
<tr>
<td>B.H.</td>
<td>63</td>
<td>907</td>
<td>30</td>
<td>10.3</td>
<td>3</td>
<td>0.46 (0.33-0.53)</td>
</tr>
<tr>
<td>I.B.</td>
<td>37</td>
<td>1389</td>
<td>30</td>
<td>11.1</td>
<td>3</td>
<td>0.28 (0.24-0.33)</td>
</tr>
<tr>
<td>S.M.</td>
<td>70</td>
<td>1134</td>
<td>31</td>
<td>9.9</td>
<td>3</td>
<td>0.54 (0.41-0.62)</td>
</tr>
<tr>
<td>B.T.</td>
<td>42</td>
<td>964</td>
<td>33</td>
<td>11.0</td>
<td>3</td>
<td>0.47 (0.38-0.58)</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>0.55 (0.45-0.67)</td>
</tr>
</tbody>
</table>

on regular formula with supplemental iron and vitamins, including vitamin E. None of the infants were jaundiced, had hepatosplenomegaly, or had evidence of renal disease.

A group of older children, ranging from 1 to 15 yr of age, followed at Children's Memorial Hospital with anemias of varied etiology and with hematocrits similar to those found in the premature infants, were also studied.

Hematologic Studies

Hematocrit and hemoglobin values were obtained on all patients using a Coulter S Electronic Cell Counter. Erythrocyte morphology was examined by peripheral blood smear in all instances.

ESF Bioassay

A sample of approximately 2.5-5 ml of blood was obtained from each infant and older anemic child by antecubital venepuncture. Serial specimens on the same infants were not drawn because of the large amount of blood required for each assay. The serum was separated and frozen at –20°C for up to 3 mo until the assay was performed.

Serum ESF levels were determined by a modification of the bioassay method of Cotes and Bangham. A sample of approximately 2.5-5 ml of blood was obtained from each infant and older anemic child by antecubital venepuncture. Serial specimens on the same infants were not drawn because of the large amount of blood required for each assay. The serum was separated and frozen at –20°C for up to 3 mo until the assay was performed. The serum was made polycythemic by being placed for 18 hr daily for 20 days in a hypobaric tank with 0.4 atmospheres pressure. Five days after removal from the tank each mouse was injected subcutaneously with 0.25 ml of either the serum to be assayed, or saline, sodium chloride as a control, or one of several concentrations of a known ESF standard preparation (ESF Step I Preparation, Connaught Laboratories, Willowdale, Ontario, Canada). These 0.25-ml injections were repeated 24 hr later. At 48 hr after the first injection, each mouse received an intraperitoneal injection of 0.2 ml of a solution containing 1.0 μCi 59Fe [ferrous citrate 59Fe, Mallinckrodt Chemical Works, St. Louis, Mo.; specific activity (Fe) 6.0 mCi/mg]. Seventy-two hours later each mouse was weighed and sacrificed. The hematocrit was determined, and 0.5-1.0 ml heparinized blood was obtained for counting. The percent incorporation of 59Fe into peripheral red cells was determined for each mouse by counting the samples in a well-type scintillation counter. Mice with hematocrits less than 53% were excluded from analysis.

For each standard, saline control, and most of the anemic control sera, five mice were injected and the mean 59Fe incorporation for the group determined. Because only 1.5 or 2.0 ml of serum could be obtained from some of the premature infants, only three or four mice were used for these
assays. A log dose-response curve for standard ESF was constructed, permitting conversion of $^{59}\text{Fe}$ incorporation in the unknown serum samples into International Units of ESF per milliliter of serum. This particular assay was not sensitive enough to detect serum ESF levels below 0.1 U/ml, and levels greater than 1.6 U/ml could not be quantitated.

**Erythropoiesis Inhibitor Assay**

In a separate experiment inhibitors against erythropoiesis were sought in the serum of two of the anemic premature infants (C.S. and N.K.). A 0.25-ml quantity of serum from each patient and 0.25 ml of a solution containing 0.1 U of ESF were injected simultaneously in two different subcutaneous sites into groups of polycythemic mice on 2 consecutive days. The percent $^{59}\text{Fe}$ incorporation was determined and compared to that for 0.2 U ESF alone in order to determine the presence of erythropoietic inhibitory substances in the test serum.

**RESULTS**

The results of the hematologic studies and ESF assays are shown in Table I and Fig. 1. There was a marked variation in the degree of anemia, with hematocrits ranging from 19% to 33% in the premature infants and from 12% to 29% in the older anemic children. In the premature infants, the hematocrit values did not correlate with birth weight, gestational age, or postdelivery age. No significant abnormalities were noted on examination of smears of peripheral blood.

No detectable ESF was found in any of the anemic premature infants’ sera (Table I and Fig. 1). However, elevated levels of ESF, from 0.13 to greater than 1.6 U/ml, were measured in the serum of all of the older children with various types of hemolytic, nutritional, and hypoproliferative anemias.

The mean $^{59}\text{Fe}$ incorporation after injection of 0.2 U ESF (4.5% ± 0.9% SEM) was not significantly diminished by simultaneous injection of the serum of C.S. (5.1% ± 0.8) or N.K. (5.3% ± 0.9). Hence, there appeared to be no inhibitory effect of these infants’ sera on erythropoiesis as measured by $^{59}\text{Fe}$ uptake into mouse red cells.

![Fig. 1. Relation between hematocrit and 72-hr $^{59}\text{Fe}$ incorporation into mouse red cells as a measure of serum ESF activity. The dotted horizontal line represents the mean $^{59}\text{Fe}$ incorporation for the saline controls.](image-url)
DISCUSSION

In the human, ESF appears to be the prime if not the sole mediator of erythropoiesis in late fetal and neonatal life. It is detected in amniotic fluid and umbilical cord blood and is elevated in both in states of intrauterine hypoxia. ESF disappears from the blood by the end of the first day of life and cannot again be detected in full-term infants until 60–90 days of age. However, during these first 90 days of life the hematocrit normally does not fall below 30% in term infants. Since a hematocrit of around 30% is generally the upper limit of detection of ESF in the bioassay, it is not surprising that ESF is not demonstrated in physiologically anemic full-term infants. However, normal premature infants, with hematocrits less than 30%, might be expected to have measurable ESF in their serum. Our finding of undetectable serum ESF in these premature infants, most with hematocrits less than 30%, suggests that decreased hormonal stimulation of the marrow erythron is a factor in the pathogenesis of their anemia.

There are several possible explanations for this seemingly impaired ESF response. One interpretation is that these infants’ tissue requirements for oxygen are minimal and are met at a relatively low hemoglobin level, such as in older patients with hypothyroidism. The data on basal metabolic rates in premature infants are conflicting, however, and do not entirely support this hypothesis. If these infants had right-shifted oxygen-hemoglobin dissociation curves, they would be able to meet tissue oxygen requirements with low hemoglobin levels and hence require less ESF. However, premature infants’ red cells have greater amounts of fetal hemoglobin and frequently lesser quantities of 2,3-diphosphoglycerate (2,3-DPG) than age-matched full-term infants. This results in a reduced P50 and a left-shifted curve. Another possibility is that there is functional immaturity of those cells in the premature infant's kidney responsible for ESF production. There is good evidence for immature glomerular and tubular function in the kidneys of normal premature infants. But other studies have shown that ESF can be produced in detectable quantities in premature infants of this age who are subjected to the intense hypoxia of severe cyanotic congenital heart disease or who are administered cobalt, an agent which induces renal ESF production. Hence the premature infant is capable of making ESF.

Inhibitors to erythropoiesis have previously been reported in full-term and premature infants up to 6 wk of age. None were found in two of our infants, but their presence cannot be entirely ruled out.

The results of this study certainly do not discriminate between the possibilities that erythropoiesis is partially or completely independent of ESF in these infants or that their marrows might be responsive to amounts of ESF below those detectable in the bioassay. Although many of these premature infants were of similar ages and birth weights, their hematocrits were markedly different. In many individual infants the hematocrit remained stable or rose during a period of weight gain. Therefore erythropoiesis did occur in these infants despite undetectable ESF, suggesting that other factors might regulate erythropoiesis or that a level of ESF below detectability was present. More sensitive measurements of ESF will be necessary to begin to resolve these possibilities.
In summary, this study demonstrates a reduction of serum ESF in premature infants with hemoglobin levels that result in elevated ESF in the serum of older children. The reason for this difference is uncertain. Diminished ESF must be considered to be normal in such infants. The low level may represent a physiologic reduction in ESF production because tissue oxygen requirements in premature infants are, in some as yet undetermined manner, satisfied at low hemoglobin levels.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. Eugene Goldwasser and Ms. Evelyn Gaston of the University of Chicago for their valuable technical assistance and to Dr. David G. Nathan for his helpful advice.

REFERENCES

24. Oski FA, Delivoria-Papadopoulos M.
The red cell, 2,3-diphosphoglycerate, and tissue oxygen release. J Pediatr 77:941, 1970
25. James JA: Renal Disease in Childhood (ed 2). St. Louis, Mosby, 1972, p 115
Impaired Erythropoietin Response in Anemic Premature Infants

George R. Buchanan and Allen D. Schwartz