The Effect of Amelioration of Anemia on the Synthesis of Fetal Hemoglobin in Sickle Cell Anemia

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Fetal hemoglobin, a tetramer of $2\alpha$ and $2\gamma$ polypeptide chains, constitutes the major hemoglobin component in newborn infants. During the first 6 months of extra-uterine life, the synthesis of $\gamma$ chains declines as $\beta$ chain synthesis increases. Adult Hb A, a tetramer of $2\alpha$ and $2\beta$ polypeptide chain accounts for over 95 per cent of the hemoglobin of most normal children at one year of age.\(^1\)

In certain hereditary disorders of hemoglobin synthesis, e.g., sickle cell anemia and thalassemia major, the synthesis of $\gamma$ chains is not completely suppressed, and appreciable amounts of fetal hemoglobin may be found in the red cells of adults with these diseases.\(^2\) In occasional patients with acquired hematologic disorders,\(^3,4\) e.g., aplastic anemia or leukemia, the synthesis of $\gamma$ chains is apparently resumed, and some erythrocytes containing Hb F appear in the circulation.

Except for individuals with the curious genetic anomaly designated “hereditary persistence of hemoglobin F”\(^5\) adults in whom Hb F synthesis persists or is renewed are frequently anemic. Many severe anemias are not associated with increased synthesis of Hb F, and a lack of correlation between the degree of anemia and the proportion of the fetal pigment has been noted in sickle cell anemia,\(^2,6\) and in aplastic anemia.\(^7\) Thus the relationships between hemoglobin concentration and persistent or renewed production of Hb F are uncertain.

The present experiments were designed to ascertain whether correction of anemia for prolonged periods would influence the proportion of Hb F in the erythrocytes of patients with sickle cell anemia.

Patients and Methods

Patients

Two adult women with sickle cell anemia and increased proportions of Hb F in their erythrocytes were studied. Hematologic data on these patients before transfusion studies were initiated and after the studies were completed are given in table 1. Electrophoresis of...
the hemoglobin of each patient on starch gels at pH 8.6⁸ and on agar gels at pH 6.2⁹ revealed Hb A₂, S, and F.

O. E., a 27-year-old Puerto Rican housewife, had increasingly frequent painful crises during 1961 and 1962. Her first symptoms of sickle cell anemia had occurred in early childhood. Both of her parents and her only child had sickle cell trait (Hb. A + S). She had received transfusions previously for sickle cell crises; the last transfusion had been given a year before the beginning of the present study. Her red blood cells were type A, Rh-positive. The patient became pregnant and the study was terminated after 28 weeks because of the appearance of the clinical and laboratory findings of hepatitis. She received intermittent transfusions of group A blood through the remainder of the pregnancy, and an apparently normal male infant was delivered by Caesarean section during the 37th week of gestation. A recurrence of hepatitis in the post-partum period was followed by slow but complete recovery.

Patient Z. T., a 36-year-old Negro woman with anemia had painful crises and recurrent leg ulcers for many years. On her initial admission to the Bronx Municipal Hospital Center in April 1962, she had an indolent ulcer at the donor site (thigh) of a skin graft, as well as an ulcer on the left leg. Her erythrocytes were type A₂B, Rh-positive. She received 20 units of blood over a period of 16 weeks, felt well and the ulcers of her thigh and lower leg healed.

Transfusions

All of the transfusions given during the study were of blood group O. Donor red cells were generally less than one week old and were administered as sedimented red cells without saline washing. The absence of Hb S in each donor blood was ascertained by electrophoresis before the transfusion was administered. Most of the maintenance transfusions were given in the out-patient clinic and 3 mild urticarial reactions constituted the only immediate reactions observed. (Patient O. E. developed hepatitis, vide supra).

Laboratory Methods

Hemolysates were prepared by the method of Drabkin.¹⁰ Fetal hemoglobin was estimated as the alkali resistant fraction by the method of Singer, Chernoff and Singer.² Electrophoresis of hemoglobin prepared from donor blood was carried out on paper or on cellulose acetate at pH 8.6. Agar gel electrophoresis at pH 6.2 for identification of Hb F was performed by a modification of the method of Robinson and associates.⁹ For estimation of Hb S and A, electrophoresis on starch gels⁸ or on starch granules¹¹ was employed.

Separation of recipient and donor erythrocytes: The patient's erythrocytes (type A₁ (O. E.) and type A₂B (Z. T.)) were separated by a modified Ashby technic¹² from the group O donor cells. The erythrocytes from 25 ml. of blood anticoagulated with EDTA were washed four times with cold saline. Washed red cells from patient O. E. were mixed with equal volumes of cold saline and of commercial anti-A blood typing serum. The mixture was allowed to stand at 4 C. for 20 minutes. The recipient's erythrocytes formed a compact “button” at the bottom of the tube, and the unagglutinated (group O) erythrocytes together with small clumps of agglutinated erythrocytes remained in the supernate. The button of agglutinated red cells was washed 7 or 8 times with cold saline by gentle inversion of the test tube with subsequent removal of the supernate. The residual colored antiserum was then removed by more vigorous dispersion of the red cells in saline followed by centrifugation. Hemolysates were prepared from the residual agglutinated erythrocytes. In the case of Z. T. (blood group A₂B), both anti-A and anti-B sera were needed to produce strong agglutination. Centrifugation at 1000 rpm for one or two minutes provided a button of agglutinated cells which were washed and hemolyzed.

During development of the technic for separation of donor and recipient red cells it was noted that smaller volumes of anti-sera failed to produce sufficiently strong agglutination. When the period of incubation was extended to 2 hours, excessive contamination of the recipient cells with donor cells was observed.
Since the erythrocytes of both recipients contained only Hb A2, S and F, the demonstration of any Hb A indicated contamination of the agglutinated cells by donor cells. Hemolysates prepared from agglutinated cells were examined by a technic of agar gel electrophoresis that could detect Hb A in excess of 5 per cent. In only a few samples of hemoglobin prepared from agglutinated recipient cells could a fine band of Hb A be demonstrated; in most of the samples Hb A was undetectable.

Separation of donor and recipient erythrocytes by differential agglutination resulted in the loss of some less strongly agglutinated recipient red cells. It was therefore necessary to demonstrate that the proportions of Hb S and F were identical in the strongly and in weakly agglutinated red cells. Red cells of two untransfused patients with sickle cell anemia were studied. Following the addition of appropriate anti-sera, the cells in the agglutinated button were separated from the cells which remained in suspension. The proportions of fetal hemoglobin in the strongly and weakly agglutinated fractions were found to be identical.

Although unagglutinated (donor) cells were not utilized in our studies, samples of donor cells with less than 2 per cent contamination by recipient cells, as determined by quantification of Hb S, could be prepared by sedimentation of the supernatant red cell suspension after agglutination of the recipient's erythrocytes. The proportion of alkali-resistant hemoglobin in the unagglutinated fraction was determined on 19 separate blood samples of patient O. E. The proportion of Hb F varied from 0.6 to 3.3 per cent with a median value of 1.3 per cent. These results are similar to the values for normals in our laboratory.

Isotope Studies

Labeling of recipient erythrocytes with Na₅CrO₄: Nine days before beginning of the transfusion study, a study of erythrocyte survival by Cr⁵¹ labeling was begun in patient Z. T. The method of Donohue and associates was employed except that red cells were incubated with 120 μc. of Na₅CrO₄ of high specific activity, and the Cr⁵¹ activity of the blood samples was expressed as net counts per mg. hemoglobin. The larger amount of Na₅CrO₄ administered, and the calculation of Cr⁵¹ activity with reference to hemoglobin concentration were designed to permit study of radioactivity in the recipient's erythrocytes after transfusions had been given.

Fe⁵⁹ incorporation studies: During the 16th week of the transfusion period, patient Z. T. was given 20 μc. of Fe⁵⁹ ferrous citrate intravenously. On the 4th, 5th 6th and 9th day after the administration of the radioactive iron, 35 ml. of venous blood were obtained. The patient's erythrocytes (group A,B) were separated from each sample as described above, and hemolysate was prepared. Hb F was isolated by the chromatographic method of Allen and associates using developer No. 2. The remaining hemoglobin was eluted from the column, and Hb S was isolated by electrophoresis of this eluate on starch granules. Fe⁵⁹ activity of cyanmethemoglobin derivatives of Hb S and F were measured, and hemoglobin concentrations were determined spectrophotometrically.

Counting of both Cr⁵¹ and Fe⁵⁹ samples was done in a well type scintillation counter. Sufficient counts were recorded to achieve an accuracy of 3 per cent.

RESULTS AND DISCUSSION

The results of serial studies carried out on patient O. E. and patient Z. T. are depicted graphically in figures 1 and 2, respectively. In patient O. E., in the control (pre-transfusion) period, values for hemoglobin concentrations were 8 to 9 Gm. per cent, with 14 to 18 per cent reticulocytes. During the first two weeks of the transfusion study, O. E. received 4 units of group O blood. At the end of the second week, her hemoglobin concentration had increased from 8.4 to 13.6 Gm. per cent, and the reticulocytes had declined to less than 2 per cent. In the ensuing 26 weeks, patient O. E. received an additional 22 units of whole blood; during the transfusion period, her
Fig. 1.—Patient O. E.: The effect of transfusions on hemoglobin and reticulocyte values and on the proportion of Hb F in the recipient's erythrocytes. Each arrow indicates transfusion of one unit of packed red cells. The bar graph below indicates the proportions of Hb A and S in hemolysates prepared from whole blood (donor and recipient cells), determined by starch block electrophoresis.11

Hemoglobin concentration varied between 11 and 14 Gm. per cent, and the reticulocyte count remained low. (Since hemoglobin concentrations were determined immediately prior to transfusions, the observed values represented the lowest values during the period of transfusion treatment.) More frequent transfusions were required to maintain desired hemoglobin concentrations during the later weeks of the study. The increased transfusion requirement might have reflected decreased survival of donor red cells as a result of undetected immunization or of the effects of hepatitis together with pregnancy.

During the 3 months prior to transfusion, patient Z. T.'s hemoglobin concentration varied between 7 and 8 Gm. per cent and the reticulocyte count ranged from 12 to 20 per cent. Patient Z. T. received as "priming" transfusions 5 units of group O erythrocytes in 3 days and the hemoglobin concentration rose to 14 Gm. per cent. For the next 15 weeks she received 2 units of blood bi-weekly and the hemoglobin concentration remained in the range of 11 to 15 Gm. per cent.

Suppression of erythropoiesis in both patients by transfusions was inferred from the results of bone marrow examinations, reticulocyte counts and quantitative determinations of Hb S and A in circulating (recipient plus donor) erythrocytes. Marked erythroid hyperplasia was observed in bone marrow
FETAL HEMOGLOBIN IN SICKLE CELL ANEMIA

Fig. 2.—Patient Z. T.: The symbols are the same as in figure 1. It should be noted that the transfusion period (abscissa) was of only 16 weeks duration.

Aspirates prior to the study. Bone marrow aspiration in each patient during the transfusion period revealed normal cellularity and a normal myeloid:erythroid ratio. Reticulocyte counts of both patients remained at decreased levels during the transfusion period; patient O. E.'s reticulocytes ranged between 1 and 4 per cent on 29 determinations and patient Z. T.'s reticulocytes ranged between 0.1 and 2.1 per cent, being less than 1 per cent on 14 of 19 determinations. However, since donor erythrocytes which contained few, if any, reticulocytes accounted for most of the circulating red cells, reticulocytes undoubtedly represented a larger proportion of the red cells of both recipients.

The most satisfactory index of suppression of erythropoiesis appeared to be quantitative determinations of Hb S and A in circulating erythrocytes (lower portion of figures 1 and 2). Hb “S” as eluted from starch blocks also contained small amounts of Hb A2 and F. Hb “S” in patient O. E. fluctuated somewhat but generally accounted for less than 20 per cent of the total hemoglobin. In patient Z. T., the proportion of Hb “S” declined gradually to about 5 per cent during the final weeks of transfusion period. Thus considerable suppression of erythropoiesis apparently occurred in both patients.

The effect of correction of the anemia by transfusions on the proportion of Hb F in the recipients' erythrocytes is shown in figures 1 and 2. Decreased hemoglobin synthesis as a result of erythropoietic suppression occurred in both patients; the studies to be described were concerned with the relative amounts of Hb S and F in erythrocytes of each recipient during the transfusion period. Since the effects of transfusions on proportions of fetal hemoglobin differed in the two patients, this aspect of the study in each patient will be discussed separately.
Table 1.—Hematologic Data on Patients O. E. and Z. T. before and after the Transfusion Study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Hb Gm. Per Cent</th>
<th>Reticulocyte Per Cent</th>
<th>Hb F (Per Cent / Total Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. O. E.</td>
<td>4/5/62</td>
<td>8.3</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4/6/62</td>
<td>8.4</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>4/14/62</td>
<td>—</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>Received regular transfusions group O blood from 4/14/62 to 10/25/62</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5/1/63</td>
<td>7.7</td>
<td>22</td>
<td>9.0*</td>
</tr>
<tr>
<td></td>
<td>5/15/63</td>
<td>7.5</td>
<td>26</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>7/8/63</td>
<td>7.8</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>7/26/63</td>
<td>7.5</td>
<td>24</td>
<td>12</td>
</tr>
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<td></td>
<td>10/9/63</td>
<td>7.9</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1/6/64</td>
<td>7.9</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Received transfusions as needed group A blood from 10/25/62 to 2/16/63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Z. T.</td>
<td>7/3/62</td>
<td>7.3</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>8/10/62</td>
<td>7.4</td>
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<td></td>
<td>9/26/62</td>
<td>—</td>
<td>—</td>
<td>12</td>
</tr>
<tr>
<td>Received regular transfusions group O blood from 9/29/62 to 1/15/63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/4/63</td>
<td>8.4</td>
<td>21</td>
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</tr>
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<tr>
<td></td>
<td>1/9/64</td>
<td>8.6</td>
<td>15</td>
<td>11</td>
</tr>
</tbody>
</table>

*Clinical hepatitis still evident at the time of this determination.

1. Patient O. E. (fig. 1)

During the control period, Hb F accounted for about 12 per cent of the total hemoglobin. After the initial transfusions, the proportion of Hb F in recipient cells increased to a peak value of 30 per cent during the fifth week and then fell to 8.5 per cent in the sixth week.

These striking alterations in the proportions of Hb F probably reflect heterogeneity of the red cells with respect to content of Hb F in sickle cell anemia. Many types of experimental evidence indicate that the cellular distribution of Hb S and F in the erythrocytes of patients with sickle cell anemia is non-uniform. The transfusion studies of Singer and co-workers,15 the heterogeneous staining properties of the red cells after acid treatment,5 the selective lysis of erythrocytes by osmotic or mechanical trauma,16 and the differences in incorporation of Fe59 into Hb S and F16 indicate heterogeneity of cellular distribution of Hb F. Therefore, in sickle cell anemia some erythrocytes may contain little, if any, Hb F while others contain rather large proportions. Erythrocytes with greater proportions of Hb F have longer survival times.15,16 Studies of patients heterozygous for both Hb S and hereditary per-
Table 2.—Radioactivity of Cr\textsuperscript{51}-Labeled Hemoglobin before and during Transfusion Study (Patient Z. T.)

<table>
<thead>
<tr>
<th>Date</th>
<th>Day of Transfusion Study</th>
<th>Cr\textsuperscript{51} Activity CPM per mg. Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/21/62</td>
<td>9 days prior to beginning of transfusions</td>
<td>33.9 (100%)</td>
</tr>
<tr>
<td>9/29/62</td>
<td>Transfusions begun</td>
<td></td>
</tr>
<tr>
<td>10/4/62</td>
<td>5</td>
<td>16.3</td>
</tr>
<tr>
<td>10/8/62</td>
<td>9</td>
<td>16.0</td>
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<td>10/11/62</td>
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<td>14.2</td>
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<td>10/18/62</td>
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<td>10/22/62</td>
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<td>10/30/62</td>
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<tr>
<td>11/6/62</td>
<td>38</td>
<td>10.9</td>
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<tr>
<td>11/16/62</td>
<td>48</td>
<td>7.8</td>
</tr>
<tr>
<td>11/30/62</td>
<td>62</td>
<td>5.8 (17%)</td>
</tr>
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</table>

Table 3.—Fe\textsuperscript{59} Incorporation into Hemoglobins S and F after Four Months of Transfusions (Patient Z. T.)

<table>
<thead>
<tr>
<th>Day after Fe\textsuperscript{59} Administration</th>
<th>Hemoglobin Component</th>
<th>Fe\textsuperscript{59} Activity CPM per mg. Hb</th>
<th>Ratio Fe\textsuperscript{59} Activity—Hb S to Fe\textsuperscript{59} Activity—Hb F</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>S</td>
<td>48.2</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>S</td>
<td>50.0</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>S</td>
<td>46.6</td>
<td>3.2</td>
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<td>F</td>
<td>14.6</td>
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<tr>
<td>9</td>
<td>S</td>
<td>28.4</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>13.1</td>
<td></td>
</tr>
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</table>

sistence of fetal hemoglobin suggest that fetal hemoglobin in proportions of 25 per cent uniformly distributed in all erythrocytes protects against sickling and hemolysis.\textsuperscript{16}

After suppression of erythropoiesis by transfusions, erythrocytes containing mostly Hb S and little Hb F were probably destroyed more rapidly than those containing higher proportions of Hb F. Recipient red cells with higher proportions of Hb F and longer survival then comprised the agglutinated fraction. The observed increase in Hb F during the first five weeks reflected decline in the Hb S "pool," with a less marked fall in the Hb F "pool." Although the possibility of increased synthesis of Hb F could not be excluded on the basis of these data, the observed rise in proportion of fetal hemoglobin was attributed to relatively prolonged survival of erythrocytes containing large amounts of Hb F.

After the maximum value for Hb F (30 per cent) was reached during the fifth week, the proportion of the fetal pigment fell abruptly and fluctuated
between 8 and 12 per cent for the next 13 weeks. During the final (19–27) weeks of the study, the proportions of Hb F (6 per cent) were significantly lower than the values (12 per cent) obtained during the control period. The longer lived cells with higher proportions of Hb F produced many weeks earlier might have contributed to the observed values for Hb F for 10 or 12 weeks. However, the presence of Hb F in the recipients cells after 4 months of transfusions was attributed to continuing synthesis of Hb F. The significant decline in Hb F after nearly 5 months of transfusions indicated that Hb F synthesis was ultimately suppressed by transfusions.

Alternatively, the lower proportion of Hb F observed late in the transfusion period might also result from prolonged survival of the patient's erythrocytes with a high content of Hb S in the presence of circulating normal erythrocytes. Direct experimental evidence concerning this possibility is not available.

Unfortunately the transfusion study in this patient was complicated by both pregnancy and hepatitis. However, pregnancy would not be expected to be associated with a decrease in synthesis of Hb F,17 and decreased proportions of Hb F were observed at least 6 weeks prior to the appearance of symptoms of hepatitis.

Thus in patient O. E. (fig. 1) fetal hemoglobin synthesis apparently continued for at least 16 weeks after correction of the anemia. After 20 weeks of transfusion therapy, the proportion of Hb F appeared to be definitely decreased when compared to the values obtained in the control period.

2. Patient Z. T. (fig. 2)

In patient Z. T., Hb F comprised 12 per cent of the hemoglobin on several determinations during the 3-month control period preceding the transfusion study. At the end of the fourth week of the transfusion period, the proportion of Hb F had risen to 17 per cent and remained in that range for the next 7 weeks. As in patient O. E., this rise in proportion of Hb F was attributed to the prolonged survival of erythrocytes containing larger amounts of Hb F in the presence of suppression of erythropoiesis.

In patient Z. T., confirmatory evidence for prolonged survival of some erythrocytes was obtained from the Cr¹¹ activity of the hemoglobin of the agglutinated erythrocytes. While the interpretation of Cr¹¹ data for erythrocyte survival during a period of suppression of erythropoiesis is difficult,18 the Cr¹¹ activity of the hemoglobin of the agglutinated cells at 70 days was 17 per cent of the activity of the hemoglobin observed 24 hours after the administration of the isotope (table 2). This study yielded direct evidence for survival of some erythrocytes, presumably those with larger amounts of Hb F, for more than 10 weeks.

After the eleventh week, the proportion of Hb F fell gradually to the control level of 11 per cent. It seemed unlikely that erythrocytes made during the control period contributed to the proportion of Hb F observed in the agglutinated cells during the 16th week. Thus continued synthesis of Hb F despite prolonged correction of anemia was also demonstrated in this patient.

Direct evidence for continuing Hb F synthesis was obtained by Fe⁵⁹ in-
corporation studies (table 3). Radioactive iron was given during the 16th week of the transfusion period, and Fe\textsuperscript{59} incorporation into Hb F and S was demonstrated on days 4, 5, 6 and 9 after the administration of the isotope. These data indicated continuing hemoglobin F synthesis. Since Hb S and F are not removed from the circulation at the same rates, the Fe\textsuperscript{59} incorporation data could not be used for quantitative determinations of synthetic rates. These data did not differ significantly from those obtained earlier\textsuperscript{16} on Fe\textsuperscript{59} incorporation into Hb S and F of a patient with sickle cell anemia who had not received recent transfusions. The progressive decline in the ratio of Fe\textsuperscript{59} activity in Hb S to Fe\textsuperscript{59} activity in Hb F probably reflected the selective destruction of labeled erythrocytes containing higher proportions of Hb S.

In table 1 values are given for the hemoglobin concentrations and proportions of Hb F for both patients several months after the conclusion of the transfusion period. Five months after the transfusion period, the hemoglobin concentration and proportion of Hb F in O. E. had returned to the pretransfusion value. Eight months after the conclusion of transfusion period, the proportion of Hb F in the erythrocytes of patient Z. T. appeared to be slightly lower than in the control period.

**COMMENTS**

The present studies indicated that many months of observation were needed for evaluation of the effect of an environmental alteration on production of Hb F in vivo. In part, this lag period represented an "unsteady state," based upon the variability of survival times of erythrocytes containing differing proportions of Hb S and F. However, in the case of O. E., the 6-month period between the initiation of transfusions and the decline in proportion of Hb F was too long to be related to survival of erythrocytes released from the marrow prior to the transfusions. The findings in patient O. E. suggested that amelioration of anemia might ultimately suppress at least partially the synthesis of Hb F.

The transfusion period of only 4 months in patient Z. T. may have been too brief to demonstrate suppression of Hb F synthesis. However, a lag in the return to pre-transfusion levels of Hb F after transfusions had been concluded seemed to occur in patient Z. T.; 4 months after transfusions were stopped, the proportion of Hb F appeared to be less than in the control period.

No observations on Hb F were made during the 4-month period after transfusions were stopped. During recovery from transfusion induced erythropoetic suppression, the Hb S and F pools were in an unsteady state, and valid interpretations of the proportions of Hb F in the circulating erythrocytes during that period would be difficult.

Attempts to interpret the significance of these long lag periods were limited by the lack of information concerning the control of Hb F synthesis in normal and in pathologic states. Baglioni\textsuperscript{19} has recently reviewed some of the evidence concerning both the switchover from synthesis of Hb F to synthesis of Hb A in the newborn period and the production of Hb F in anemic individuals. He concluded that postulated mechanisms based upon alterations in oxygen
tension or in hormonal state were unsatisfactory and suggested that Hb F was found in red cells produced by few cell divisions from stem cells and that normal adult red cells produced from erythroblasts by many cell divisions would contain Hb A rather than Hb F. Since anemia is the commonest stimulus for rapid differentiation of erythrocytes from stem cells, Baglioni suggested that increased proportions of Hb F would be expected in severe anemia.

However, none of the current hypotheses concerning the mechanism of Hb F production fully account for the clinical observations on persistence or reactivation of γ chain synthesis in severe anemias. Hb F may be absent in many severe anemias with erythroid hyperplasia and may be found for many months after amelioration of the anemia in pernicious anemia,4 in aplastic anemia7 and in the present studies, in sickle cell anemia.

The changes observed in the present study suggest that anemia may be one of the factors regulating Hb F synthesis in vivo. Since in most diseases with elevated proportions of Hb F the fetal pigment is heterogeneously distributed in the erythrocyte population (vide supra), differing amounts of fetal hemoglobin are apparently synthesized in different normoblasts. Correction of the anemia probably does not affect the proportion of fetal hemoglobin ultimately synthesized in cells which have differentiated to the normoblast stage, but rather affects the activation or de-repression of Hb F synthesis in certain early precursors of the erythroid series. Normoblasts derived from these precursors may synthesize hemoglobin F for long periods after the “de-repressor” stimulus has disappeared. Activation or de-repression of Hb F synthesis in such early precursors would account for the long lag periods observed in the present study, as well as for the continuing presence of Hb F after amelioration of the anemia in pernicious anemia and in aplastic anemia.

SUMMARY

Two adult patients with sickle cell anemia of blood group A and A2B respectively, received sufficient transfusions of group O blood to maintain nearly normal hemoglobin concentrations for 4 months or longer.

Serial samples of the erythrocytes of each recipient were obtained by agglutination with anti-A (and anti-B) serum. The proportion of Hb F in the agglutinated erythrocytes was determined. Early in the transfusion period, a marked rise in the proportion of Hb F was noted. This rise was attributed to prolonged survival of erythrocytes which contained larger proportions of Hb F. In the later part of the transfusion period, the proportion of fetal hemoglobin declined to pre-transfusion levels or below. However, significant amounts of fetal hemoglobin in the erythrocytes of each patient were demonstrated throughout the period of study, and Fe²⁺ incorporation into Hb F in vivo was demonstrated in one patient after 4 months of transfusion therapy. Under the conditions of these studies, synthesis of Hb F continued despite prolonged correction of the anemia. A decline in the proportion of Hb F in the erythrocytes of one patient after 5 months of transfusions suggested that Hb F synthesis may ultimately be depressed by transfusions. It was suggested that the proportion of fetal hemoglobin observed in the erythrocytes might in certain diseases reflect the degree of anemia present many months before.
FETAL HEMOGLOBIN IN SICKLE CELL ANEMIA

SUMMARIO IN INTERLINGUA

Duo adulto patienes con anemia a cellulas falciforme del gruppcs sanguinee A e A2B recepeva sufficiente transfusiones de sanguine de gruppo O pro manten quasi normal concentraziones de hemoglobina durante 4 menses o plus.

Specimens serial del erythrocytos de cat un del recipientes esseva obtenite per agglutination con sero anti-A e anti-B. Le proportion de hemoglobina fetal in le agglutinate erythrocytos esseva determinate. Preciocemente in le curso del periodo de transfusiones un marcate augmento in le proportion de hemoglobina F esseva notate. Iste augmento esseva attribuite al prolongate supervivencia de erythrocytos que contineva plus grande proportiones de hemoglobina F. Plus tarde durante le periodo del transfusiones, le proportion de hemoglobina fetal declinava al niveles de ante le transfusiones o infra illos. Tamen, significative quantitates de hemoglobina fetal in le erythrocytos del duo patienes esseva demonstrate durante le periodo total del studio, e le incorporation de Fe\(^{3+}\) ad in hemoglobina fetal in vivo esseva demonstrate in un del patienes post quatro menses de therapia transfusional. Sub le conditiones de iste studios, le synthese de hemoglobina fetal continuava in especto de un prolongate correcticn del anemia. Un declino in le proportion de hemoglobina fetal in le erythrocytos de un del patienes post cinque menses de transfusiones suggereva que le synthese de hemoglobina fetal es forsan ultimemente deprimible per medio de transfusiones. Es signalate le possibilite que le proportion de hemoglobina fetal observe in le erythrocytos de un date patiente reflecte in certe morbos le grado de anemia que esseva presente multe menses retro.

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The Effect of Amelioration of Anemia on the Synthesis of Fetal Hemoglobin in Sickle Cell Anemia

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