Studies on the Fetal Hemoglobin in the Persistent High Hb-F Anomaly

By R. B. Thompson, J. W. Mitchener and Titus H. J. Huisman

It is well-known that fetal hemoglobin (Hb-F) occurs in large quantities in the blood of newborn children. It almost completely disappears within some months after birth although its synthesis at a low level during adult life is responsible for small quantities of Hb-F (about 0.5 per cent) in the blood of normal adults. Hb-F may reappear in abnormal amounts in various pathologic conditions. Increased values of Hb-F in postnatal life also are found associated with the abnormal hemoglobins S, C and E (Hb-S, Hb-C, Hb-E) in certain hereditary hematologic disorders in which genes for thalassemia, sickle-cell hemoglobin, or combinations of the thalassemia gene and the gene regulating the production of an abnormal hemoglobin are present. In thalassemia major, for instance, the amount of Hb-F is increased to 20 to 90 per cent while in the heterozygous form of this condition, thalassemia minor, the level of Hb-F is normal or slightly elevated. In homozygous sickle-cell disease, the level of Hb-F may be as high as 20 per cent. The combination of a gene producing an abnormal hemoglobin and the thalassemia gene leads to a condition in which the formation of Hb-A is suppressed to various degrees, the abnormal hemoglobin dominates, but varying proportions of Hb-F coexist.

In 1955, Edington and Lehmann reported the cases of two healthy adults in Africa who appeared to be homozygous for the sickle-cell hemoglobin gene but were free of any demonstrable disease. Both had high percentages of Hb-F. Persistence of Hb-F in the adults of two African families was also reported by Jacob and Raper in 1958. Their detailed genetic and hematologic studies excluded the presence of thalassemia as well as homozygous sickle-cell anemia. The hereditary persistence of fetal hemoglobin in adult life seems to be a genetic entity different from any other hemoglobin abnormality.

When this abnormality occurs in individuals who are also heterozygous for the gene regulating the production of Hb-S ("FS combination"), only Hb-S and Hb-F are demonstrable and normal Hb-A is completely absent. This fact may add support to the genetic evidence that the gene responsible for the persistent production of Hb-F in adult life is allelic with the genes regulating the formation of Hb-A and the abnormal Hb-S and Hb-C.

From the Departments of Pathology and Biochemistry, Medical College of Georgia, Augusta, Georgia.

This investigation was supported by a U.S.P.H.S. Research Grant, H-5168. Dr. Thompson and Dr. Mitchener were supported by a U.S.P.H.S. Graduate Training Grant, 2G-207.

Submitted Apr. 21, 1961; accepted for publication June 18, 1961.

267
McCormick and Humphrey\textsuperscript{22} have recently described the combination of the persistent high Hb-F anomaly and Hb-C. This condition was found to be similar to that of the FS combination. Unfortunately the method used by these investigators to include the presence of Hb-A in the FC combination is open to some criticism.

The present report concerns the hereditary persistence of Hb-F in conjunction with normal Hb-A or Hb-S in the adults of three generations of a Negro family. The genetic data presented may support the hypothesis that the persistent high Hb-F anomaly is allelic with the genes responsible for Hb-A, Hb-S and Hb-C. Chemical studies of the isolated Hb-F from the adults show that this hemoglobin is most likely identical with the fetal hemoglobin of the newborn infant. Observations are also reported on the distribution of the Hb-F in the erythrocytes of different cases in which high percentages of Hb-F is present in addition to Hb-A and/or an abnormal adult hemoglobin. Part of this study has been presented earlier.\textsuperscript{27}

**CASE REPORT**

Some five days before admission to our hospital, a 14 year old colored male (M. P., Medical College of Georgia, Eugene Talmadge Memorial Hospital No. 024-547) was seen by his physician and placed in his local hospital because of priapism. He became jaundiced and developed high fever while in that hospital. At the time of referral and admission to our hospital, the patient still had a painful, tender, inflamed, erected penis. He also complained of pain in the extremities, abdominal tenderness, and a sore throat. The oral temperature was 101.2 F. The red blood cells sickled promptly in a wet preparation,\textsuperscript{19} the hemoglobin was 7.2 Gm. per 100 ml., total bilirubin was 1.1 mg. per 100 ml., and the sclerae were icteric. The white blood cell count was 37,000.

From the age of two years, the patient had had similar bouts of illness lasting several days to a week and occurring about once a year. The attacks were marked by pain low in the back and extremities, sore throat, fever, and jaundiced sclerae. His parents stated that he had never been strong and tired more easily than his contemporaries.

During the ten-day course in our hospital, the diagnosis of homozygous sickle-cell disease was confirmed by extensive studies of his hemoglobin. Two 1000 ml. transfusions of whole blood caused the hemoglobin to rise to 9.9 Gm. per cent. The patient's temperature fluctuated between 99 and 105 F. for four days, and Staph. aureus, coagulase-positive, was cultured from the throat. The patient received massive doses of penicillin and chloromycetin. He also was treated with intravenous fibrinolysin and diethylstilbestrol for priapism.

After the patient's discharge from the hospital, we carried out a complete study of his family in their home community. The immediate family of the patient included three generations: grandfather and grandmother, both aged 60 years, the mother aged 28 years, the father aged 38 years, a brother aged 12 years and two female siblings aged 10 and 1 years. With the exception of the patient, all members of this family had enjoyed good health and had had no serious illnesses. We studied as controls normal adults and normal children from the same kindred. Blood samples of the three authors were used as controls for some special studies. Through the kind cooperation of Drs. C. C. de Silva (Ceylon) and K. Punt (the Netherlands), we were also able to study the blood of eight patients with thalassemia major and thalassemia-Hb-E-disease. The amounts of Hb-F in these samples varied from 12 to 48 per cent. The samples were specifically used in the investigation of Hb-F distribution in erythrocytes.

**METHODS**

A. **Hemoglobin studies:** Hemolysates of saline-washed red blood cells were subjected to electrophoresis on starch gel at pH 8.0 according to the method described by Smithies\textsuperscript{29}
and adapted to hemoglobin analysis in a previous study. Percentages of alkali-resistant hemoglobin were determined using the spectrophotometric method of Jonxis and Visser. The solubility of the hemoglobin samples in the reduced state was studied by the method of Itano. In a few cases, complete salting-out curves of the reduced hemoglobins were determined following the technic outlined by Derrien. Chromatographic analyses were carried out by the carboxymethyl-cellulose (CMC) procedure previously described; the percentages of Hb-A2 were determined by a simplified DEAE-cellulose chromatographic technic, which was recently described in detail.

B. Hematologic studies: Conventional methods were employed for determination of total hemoglobin, hematocrit, red blood cell and reticulocyte counts, and serum bilirubin. Stained smears of peripheral blood were studied. The method of Landers and Zak was used for the determination of serum iron. Osmotic resistance of the red blood cells was determined by using dilutions of NaCl ranging from 0.24 to 0.60 M per cent; the amounts of hemoglobin present in the centrifuged hemolysates were measured at 540 μm using the Beckman DU-Spectrophotometer. The method of Van Kampen, Graafland and Hasselman was used to determine the pH resistance of erythrocytes.

Since in vivo determinations were not possible, attempts were made to determine the red blood cell survival in vitro by using the method of Van Kampen and collaborators. This method, which is based upon the avidity of the red blood cells for radioactive chromium (Cr51), was applied to the blood samples of five members of the family studied and to three normal adults (the authors). Exactly 5 ml. of homogenized blood were mixed with 1 ml. Na2Cr51 O4 solution isotonic with 0.9 per cent NaCl; 1 ml. gave 8100 ± 100 cpm. with a background of 120 cpm. The mixture was incubated for one hour at 37 C. After 25 mg. Na-ascorbate were added, the total activity was measured. The activity present in the erythrocytes was measured after the cells were washed three times with 0.9 NaCl and hemolysed with saponin. Since the uptake of radioactive chromium was found to be related to the number of red blood cells, the values obtained were expressed in per cent Cr51 uptake per 10^6 RBC/mm3. Because data were insufficient to correlate in vivo and in vitro experiments, no attempts were made to calculate the half-lifetimes of the erythrocytes. However, the results obtained by the originators of this procedure showed good agreement between the increase of Cr51 uptake and the shortening of the red blood cell survival time.

The demonstration of fetal hemoglobin in red blood-cells was carried out by following the technic described by Betke and collaborators, which was introduced in our laboratory by Dr. Betke.

C. Characterization of the “fetal” hemoglobin: The abnormal, fetal-like component was isolated from the hemolysates of blood samples of four family members with the persistent high Hb-F anomaly by using 60 x 0.9 cm. CM-cellulose chromatographic columns, 0.01 M phosphate buffers, and a pH gradient previously described. The isolated fraction was concentrated by CMC-Chromatography and next studied by starch-gel electrophoresis, by rechromatography on CMC and by denaturation against alkali. Its ultraviolet absorption spectrum was determined using the Beckman DK-2-spectrophotometer. Parts of the isolated Hb-F fractions were converted to globin and separated into two polypeptide chains by following the chromatographic procedure of Wilson and Smith. The nature of the polypeptide chains was determined by establishing the elution molarities of urea, which are considered specific for the α, β, and γ polypeptide chains of human hemoglobins. Parts of the isolated Hb-fractions were also studied by the “fingerprint” technic developed by Ingram. The ninhydrin stain and the color reactions specific for various amino acids (histidine, arginine, tyrosine and tryptophane) were carried out as described in the handbook of Block, Durnim and Zweig. In all instances, fetal hemoglobin of cord blood purified by CMC chromatography was used as control.

RESULTS

A. Hemoglobin analysis and genetic studies: The results of the starch-gel electrophoresis are outlined in figure 1. In the blood of the grandfather (1,4)
and the brother (III,2) two major components were found, one with a mobility like normal Hb-A and one with a mobility similar to that of Hb-F. The grandmother (I,3), the mother (II,3) and her daughter (III,4) were heterozygous for sickle-cell hemoglobin. In the blood of the father (II,2), a large amount of Hb-S was detected while the faster moving component also present had the same mobility as fetal hemoglobin. No normal Hb-A was detectable in this sample. The 14 year old boy (III,1) whose hospital admission instigated this investigation was homozygous for sickle-cell hemoglobin although the sample used in the starch-gel electrophoresis presented in figure 1 was one taken following a blood transfusion. The ten year old sister (III,3) was normal; neither Hb-S nor fetal hemoglobin was detected in her blood.

The study of the family was extended to include all relatives living in the area. The pedigree of this entire family is presented in figure 2. Nineteen carriers of the persistent high Hb-F anomaly and one case of the FS combination

Fig. 1.—Starch gel electrophoretic pattern at pH 8.0 of the hemoglobin of the grandparents (I,3 and I,4), the parents (II,2 and II,3) and the four children (III,1, 2, 3, and 4) of the family P.
FETAL HEMOGLOBIN IN HIGH Hb-F ANOMALY

Fig. 2 — The pedigree of family P. The immediate relatives of the patient with sickle-cell anemia are represented in the stippled section.
were discovered. Extensive studies of the fetal-like component present in the blood of several members of this family will be presented later. They offer evidence that this Hb-fraction is identical with the Hb-F present in cord blood. It, therefore, will be referred to as Hb-F. The results of the determination of the solubility of the reduced hemoglobins of different members of the family showed a decreased solubility in the cases I,3, II,3, II,2, III,1 and III,4, confirming the presence of Hb-S. Complete salting-out curves were carried out for the cases II,2 and III,1. The results closely approximated those mentioned above; in case II,2 a component with a higher solubility, presumably Hb-F, was demonstrated.

The percentages of the different hemoglobin fractions as present in the blood of several members of the family were determined by CM-cellulose and DEAE-cellulose chromatography and by the spectrophotometric alkali denaturation procedure. The mean value for Hb-F in 19 cases of the persistent high Hb-F anomaly obtained by the alkali denaturation technic was 32 ± 3 per cent with a range of 23 to 38 per cent. The amount of Hb-F in the patient with sickle cell anemia (III,1) was as low as 3 per cent. The amount of Hb-F in case II,2, having the hemoglobin phenotype FS, ranged from 33 to 35 per cent. The values of fetal hemoglobin obtained by the alkali denaturation technic compared closely with those obtained by CMC chromatography. With this chromatographic technic, no normal Hb-A was detectable in the FS combination (case II,2); in addition to Hb-F, a large amount of Hb-S and a small percentage of Hb-A2 were present. Also of special interest is case III,3: in the blood of this daughter only normal Hb-A was detectable while neither the amount of Hb-F nor the per cent of Hb-A2 was increased.

The simplified DEAE-cellulose chromatographic technic was used for the determination of the Hb-A2 values. The data obtained for the several family members with the persistent high Hb-F anomaly and the FS combination are listed in table I. These are compared with the values found in normal individuals and in cases of thalassemia minor and major. The Hb-A2 values in the carriers of the high Hb-F anomaly and also in the FS combination was found to be significantly lower than normal (P-value < 0.05). They were different from the expected high levels observed in the patients with thalassemia minor and also from those found in two patients with thalassemia major. It is worth noting that the levels of Hb-F in the two patients with thalassemia major were 45 and 48 per cent respectively.

The immediate family of the patient (III,1) is represented in the stippled

---

**Table 1.**—Hb-A2 Values as Determined by DEAE Cellulose Chromatography

<table>
<thead>
<tr>
<th>Case</th>
<th>n</th>
<th>Hb-A2 (%)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>34</td>
<td>2.1 ± 0.2</td>
<td>1.6–2.5</td>
</tr>
<tr>
<td>High Hb-F anomaly</td>
<td>19</td>
<td>1.7 ± 0.2</td>
<td>1.3–2.3</td>
</tr>
<tr>
<td>FS combination (case II,2)</td>
<td>1</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Normal (case III,3)</td>
<td>1</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Thalassemia minor</td>
<td>15</td>
<td>4.4 ± 0.3</td>
<td>3.8–5.2</td>
</tr>
<tr>
<td>Thalassemia major</td>
<td>2</td>
<td>3.0; 3.3</td>
<td></td>
</tr>
</tbody>
</table>
area of figure 2. It is in this group that the interaction of the genes for Hb-S and the persistent high Hb-F anomaly occurred. The grandparents of the patient were carriers of the sickle cell trait (I,3) and the persistent high Hb-F anomaly (I,4). Their son (II,2) inherited both abnormalities; starch gel electrophoresis of his hemoglobin showed only Hb-S and Hb-F. Four children resulted from his marriage with a Hb-S trait carrier (II,3). Of these four children, one is homozygous for Hb-S, one is a heterozygous carrier of this abnormal hemoglobin, one is heterozygous for the persistent high Hb-F anomaly and one is entirely normal. Of considerable interest is the finding of a normal child as a result of a marriage between a sickle cell trait carrier (II,3) and a FS combination (II,2). Non-paternity was strongly denied. The findings for the different blood groups, however, showed a discrepancy for the S and s antigens (table 2), which makes the paternity doubtful. The difference in the Leα factor, namely a Leα positive child of two Leα negative parents, does not exclude paternity since this is a normal and fairly common finding. An interesting abnormality in the Kidd system was discovered in the father. Abnormalities in both Jk antigens were present [marked in table 2 with (+)]. The abnormality in the Jkα antigen was also found in case III,3; this finding, however, does not necessarily support paternity, since a relative, carrying the same blood group abnormality, could also be responsible for the transmission of this abnormality to case III,3. It is worth noting that these abnormalities in the Kidd system were also present in case III,1 [Jkα−, Jkβ (+)] and in case III,2 [Jkα (+), Jkβ−]. Extensive studies on the characterization and inheritance of these abnormalities are in progress.

B. Hematologic studies: The results of the hematologic studies of the patient and his immediate relatives are presented in table 3. They are compared with those obtained for normal individuals from the same area. The data obtained for the cases I,4 and III,2 (persistent high Hb-F carriers), for I,3 and II,3 (sickle-cell trait) and for III,3 (normal) are similar to those of normal controls. Case III, 1 (homozygous S) showed a low total hemoglobin, 8.4 Gm. per cent, and a low packed-cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), but a normal mean corpuscular hemoglobin concentration (MCHC). The peripheral smear showed sickling, marked hypochromia, anisocytosis and poikilocytosis of the red blood cells. The reticulocyte count and the bilirubin were elevated. Case III, 4, a female aged one year, had a low total hemoglobin, 6.9 Gm. per cent, and a low PCV, MCH and MCHC. The serum iron in this child was decreased; the peripheral smears showed marked hypochromia and microcytic cells. It seems, therefore, that her anemia was caused by iron or nutritional deficiency superimposed on sickle-cell trait. Of particular interest is case II,2 which is heterozygous for Hb-S and for persistent high Hb-F anomaly. Apart from the low

<table>
<thead>
<tr>
<th>Case</th>
<th>D</th>
<th>C</th>
<th>E</th>
<th>C</th>
<th>K</th>
<th>Fyα</th>
<th>s</th>
<th>P</th>
<th>Leα</th>
<th>Jkα</th>
<th>Jkβ</th>
<th>MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>II,2 (father)</td>
<td>O-Rh</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>II,3 (mother)</td>
<td>O-Rh</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>III,3 (daughter)</td>
<td>O-Rh</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
</tr>
</tbody>
</table>
### Table 3.—Hematologic Data Pertaining to Members of Family P and to Some Normal Controls

<table>
<thead>
<tr>
<th>Case</th>
<th>Relation</th>
<th>Age &amp; sex</th>
<th>Hb-Haplophenotype</th>
<th>RBC p. mm.(^2) x 10(^6)</th>
<th>Hb Gm. %</th>
<th>P.C.V. %</th>
<th>M.C.V. (\mu)</th>
<th>M.C.H. (\gamma)</th>
<th>M.C.H.C. %</th>
<th>Bilirubin mg. %</th>
<th>Serum Fe (\gamma) %</th>
<th>Retic. %</th>
<th>Sickle-cell formation</th>
<th>Solubility red. Hb*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I,4</td>
<td>grandfather</td>
<td>60 ♂</td>
<td>AF</td>
<td>4.22</td>
<td>14.8</td>
<td>47</td>
<td>111</td>
<td>35</td>
<td>32</td>
<td>0.1</td>
<td>105.0</td>
<td>1.0</td>
<td>-</td>
<td>84.5</td>
</tr>
<tr>
<td>I,3</td>
<td>grandmother</td>
<td>60 ♀</td>
<td>AS</td>
<td>3.54</td>
<td>11.5</td>
<td>38</td>
<td>107</td>
<td>32</td>
<td>30</td>
<td>0.3</td>
<td>125.0</td>
<td>0.1</td>
<td>+</td>
<td>55</td>
</tr>
<tr>
<td>II,3</td>
<td>mother</td>
<td>28 ♀</td>
<td>AS</td>
<td>3.35</td>
<td>12.4</td>
<td>39</td>
<td>116</td>
<td>37</td>
<td>32</td>
<td>0.2</td>
<td>200.0</td>
<td>0.2</td>
<td>+</td>
<td>62</td>
</tr>
<tr>
<td>II,2</td>
<td>father</td>
<td>38 ♂</td>
<td>FS</td>
<td>5.26</td>
<td>13.9</td>
<td>40</td>
<td>76</td>
<td>27</td>
<td>35</td>
<td>0.5</td>
<td>115.0</td>
<td>0.2</td>
<td>±</td>
<td>42</td>
</tr>
<tr>
<td>III,1</td>
<td>patient</td>
<td>14 ♀</td>
<td>SS</td>
<td>4.20</td>
<td>8.4</td>
<td>27</td>
<td>64</td>
<td>20</td>
<td>31</td>
<td>3.8</td>
<td>260.0</td>
<td>20</td>
<td>+++</td>
<td>44.5</td>
</tr>
<tr>
<td>III,2</td>
<td>brother</td>
<td>13 ♂</td>
<td>AF</td>
<td>3.70</td>
<td>11.2</td>
<td>31</td>
<td>84</td>
<td>30</td>
<td>36</td>
<td>0.3</td>
<td>130.0</td>
<td>0.1</td>
<td>-</td>
<td>87</td>
</tr>
<tr>
<td>III,3</td>
<td>sister</td>
<td>10 ♀</td>
<td>AA</td>
<td>-</td>
<td>12.4</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>35.0</td>
<td>195.0</td>
<td>0.1</td>
<td>-</td>
<td>86</td>
</tr>
<tr>
<td>III,4</td>
<td>sister</td>
<td>1 ♀</td>
<td>AS</td>
<td>3.50</td>
<td>6.9</td>
<td>29</td>
<td>83</td>
<td>20</td>
<td>24</td>
<td>0.5</td>
<td>65.0</td>
<td>1.0</td>
<td>+</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Control I</td>
<td>58 ♀</td>
<td>AA</td>
<td>4.06</td>
<td>10.9</td>
<td>35</td>
<td>86</td>
<td>27</td>
<td>31</td>
<td>0.4</td>
<td>110.0</td>
<td>-</td>
<td>-</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Control II</td>
<td>27 ♀</td>
<td>AA</td>
<td>4.01</td>
<td>11.1</td>
<td>37</td>
<td>92</td>
<td>28</td>
<td>30</td>
<td>0.5</td>
<td>95.0</td>
<td>0.1</td>
<td>-</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Control III</td>
<td>13 ♀</td>
<td>AA</td>
<td>4.40</td>
<td>12.4</td>
<td>40</td>
<td>91</td>
<td>28</td>
<td>31</td>
<td>0.5</td>
<td>160.0</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Control IV</td>
<td>11 ♀</td>
<td>AA</td>
<td>4.22</td>
<td>11.7</td>
<td>37</td>
<td>88</td>
<td>28</td>
<td>32</td>
<td>0.1</td>
<td>200.0</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Control V</td>
<td>10 ♂</td>
<td>AA</td>
<td>4.93</td>
<td>12.2</td>
<td>37</td>
<td>75</td>
<td>25</td>
<td>33</td>
<td>0.5</td>
<td>160.0</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Control VI</td>
<td>9 ♂</td>
<td>AA</td>
<td>3.99</td>
<td>11.1</td>
<td>33</td>
<td>82</td>
<td>28</td>
<td>34</td>
<td>0.4</td>
<td>150.0</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*According to Itano,\(^{19}\) expressed as % Hb remaining in solution.
M.C.V., the hematologic findings in this man were normal. Results of the conventional sickle-cell preparation and the solubility of reduced hemoglobin differed. It was difficult to obtain sickling in a preparation of the blood from case II,2, even though two-thirds of the hemoglobin was Hb-S.

Studies of the hemoglobin levels, packed cell volumes and red blood cell counts have been extended to include 19 carriers of the persistent high Hb-F anomaly and 22 normal controls, all in the same kinship. It was found that these three values in both groups bore the accepted normal relationship from one to another. However, these three values were found to be slightly higher in the persistent high Hb-F carrier than in normal controls. The mean value for total hemoglobin for 19 persistent high Hb-F carriers was 13.0 ± 1.4 Gm. per cent, while the mean value for 22 normals was 12.0 ± 1.7 Gm. per cent. It is worth mentioning that the difference seems to be most marked in the younger age group.

The osmotic resistance and pH-resistance curves of the blood samples obtained from four members of the family and from some controls are presented in figure 3. These data are also tabulated in table 4 together with those obtained for other members of the family and with the results of the studies of the in vitro Cr³⁺ uptake. The osmotic resistance of the erythrocytes of the cases I,4, II,2 and III,2 was increased; the inherited persistent high Hb-F anomaly is present in all three cases. The red blood-cells of case III,1 (homozygous Hb-S) showed a decreased resistance to hemolysis, while the range of hemolysis was increased. The results for the other members of the family fell within
normal limits. The pH resistance curves showed a close correspondence with those obtained by using salt resistance. Again the cases II,2 and III,2 differed from normal cases in an increased resistance of the erythrocytes to an acid pH. The curve found for case III,1 was again completely different from that of case II,2. The red blood cells of this patient with sickle-cell anemia were less resistant to acid pH while the range of hemolysis also was increased.

The Cr$^{51}$ uptake was studied in two cases with sickle-cell trait (I,3 and II,3) in the case of sickle-cell anemia (III,1), in a persistent high Hb-F carrier (III,2), in the father with a heterozygosity for Hb-S as well as for the persistent high Hb-F anomaly (II,2), and in three normal individuals. When expressed as per cent Cr$^{51}$ uptake per 10$^6$ RBC in 1 mm.$^3$ the values obtained for three normal adults were 12.5, 13.1 and 13.9. The per cent uptake in the cases I,3, II,3, II,2 and III,2 was only slightly higher, 14.0–16.0, while in case III,1 it was definitely higher, 29.6. It is possible that the lower total RBC count in case III,1 contributed to some extent to the higher per cent uptake of Cr$^{51}$. If an increased uptake in vitro is directly related to a decreased survival of the red blood cells, one may conclude that the presence of the persistent high Hb-F anomaly does not significantly interfere with the life-span of the erythrocytes. It seems, therefore, that the survival time of the father’s erythrocytes is normal, but that of the son’s, decreased.

Examples of the results obtained with the F-cell procedure of Betke et al.$^{24}$ are presented in figure 4. Photographs A and B were prepared to compare the cells from umbilical-cord blood, adult blood and the patient with sickle cell anemia (III,1) with 3 per cent Hb-F. A is a mixture of equal parts of cord blood containing 80 per cent Hb-F and normal adult blood; B, the blood of patient III,1. In A, two distinct cell-populations are seen: the adult cells appear as ghost forms with only a cell margin while the cord blood cells show cytoplasmic coloration. Variation in the intensity of staining of the cord blood cells demonstrates some variation in the amounts of fetal hemoglobin they contain. In figure 4B, most cells appear as ghost cells, while a few distinct acid resistant erythrocytes were also present. It will be noted that the blood smears of sickle-cell anemia patients with higher per cent of Hb-F (8–15 per cent) showed larger numbers of acid resistant red blood cells.$^{27}$ Figures 4C and D were prepared from the blood of case II,2 that is heterozygous for Hb-S as well as for the persistent high Hb-F anomaly (Hb-F—33 per cent). In C the blood of case II,2 was used alone; in D, a mixture of equal parts of normal adult blood, cord blood and blood of case II,2. One cell-population of rather uniform intensity is seen in C. In D three cell-populations can be observed: the dark staining cord blood cells, the ghost cells of normal blood and the erythrocytes of case II,2. Similar results were obtained for the blood of several carriers of the persistent high Hb-F anomaly. Figures 4E and F are examples of the results obtained by studying blood samples of patients with the thalassemia abnormality. Figure 4E was prepared from blood of a patient with thalassemia major with 45 per cent Hb-F and figure 4F from blood of a patient with Hb-E thalassemia disease with 43 per cent Hb-F. Two distinct cell populations are seen: cells appearing as ghost forms with only a cell margin, and
Table 4.—The Results of Some Special Hematologic Studies

<table>
<thead>
<tr>
<th>Case</th>
<th>Osmotic resistance NaCl in Gm./100 ml.</th>
<th>pH-resistance</th>
<th>%5CR-uptake</th>
<th>Hb-phenoype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10%</td>
<td>50%</td>
<td>&gt;90%</td>
<td>&lt;10%</td>
</tr>
<tr>
<td></td>
<td>NaCl in mm.</td>
<td>% Uptake</td>
<td>pH</td>
<td>mm.</td>
</tr>
<tr>
<td>1.4</td>
<td>0.30</td>
<td>0.32</td>
<td>0.28</td>
<td>4.88</td>
</tr>
<tr>
<td>1.3</td>
<td>0.48</td>
<td>0.40</td>
<td>0.32</td>
<td>5.57</td>
</tr>
<tr>
<td>1.2</td>
<td>0.58</td>
<td>0.42</td>
<td>0.32</td>
<td>4.46</td>
</tr>
<tr>
<td>1.1</td>
<td>0.58</td>
<td>0.42</td>
<td>0.32</td>
<td>3.78</td>
</tr>
<tr>
<td>1.0</td>
<td>0.58</td>
<td>0.42</td>
<td>0.32</td>
<td>0.58</td>
</tr>
<tr>
<td>Mean of controls</td>
<td>0.50</td>
<td>0.46</td>
<td>0.36</td>
<td>4.88</td>
</tr>
</tbody>
</table>

| Mean of 4 controls | 0.50 | 0.46 | 0.36 | 4.88 | 4.58 | 4.40 | 4.09 | 57.3 | 14.0 | AA |
| Control I          | 4.12 | 58.7 | 13.1  |
| Control II         | 4.11 | 57.5 | 13.9  |
| Control IV         | 4.58 | 56.2 | 12.5  |

It seems, therefore, that the fetal hemoglobin in the persistent high Hb-F carrier is present in every cell and in about the same amounts, while specific fetal hemoglobin-containing cells are present in the thalassemia abnormality.

C. Studies on the isolated fetal hemoglobin: The fetal hemoglobin of four cases with the persistent high Hb-F anomaly, isolated and purified by CMC chromatography, was characterized by the use of different analytic procedures. In all instances, the results were compared with those found for the fetal hemoglobin of cord blood isolated in exactly the same way. Both hemoglobin components behaved identically in starch gel electrophoresis and CMC chromatography. Their alkali denaturation curves were the same and the curves representing their ultraviolet spectral absorption also were identical. The fetal hemoglobin was separated into two polypeptide chains by using the Amberlite IRC-50 chromatographic procedure. The elution molarity of urea for the first polypeptide chain, 5.7 M, was the same as that found for the a chain of normal adult and fetal hemoglobin, 5.8 ± 0.1 M. The elution molarity of the second polypeptide chain, 6.6 M urea, indicated a similarity with the y chain of the Hb-F from cord blood which has a value of 6.7 ± 0.1 M contrasted with 6.9 ± 0.1 M for B-chain of Hb-A. The results of the study for possible structural abnormalities is given in figure 5. The tracings of the "fingerprints" of Hb-F of family P and of Hb-F from cord blood presented in this figure demonstrate no notable difference between the tryptic digests of these two proteins. Also, the stains specific for some amino acids (histidine, arginine, tyrosine and tryptophane) failed to show a significant difference between the peptides present in the two protein digests. Our "fingerprints" of Hb-F are almost identical with those described by Hunt.

Discussion

A family study centering around a case of homozygous sickle-cell disease uncovered several family members with fetal hemoglobin fractions as high as
Fig. 4.—The demonstration of fetal hemoglobin in the red blood cells following the technic of Betke et al.24 The following blood samples were used: (A) mixture of equal amounts of cord blood and of blood of a normal adult; (B) patient III,1, homozygous Hb-S with 3 per cent Hb-F; (C) case II,2; (D) mixture of equal amounts of cord blood, blood of normal adult and blood of case II,2; (E) thalassemia major (45 per cent Hb-F); (F) thalassemia-Hb-E disease (43 per cent Hb-F). Pictures were taken with a Zeiss photomicroscope, using 35 mm. Panatomic X film; developed in Microdol. Photographs were printed on Kodak enlargement paper, developed in Dektol.

23 to 38 per cent. In 19 cases, normal Hb-A was demonstrated with Hb-F and in one case only Hb-S and Hb-F. The fetal hemoglobin was identical with the fetal hemoglobin of the newborn infant. Both were identical in electrophoretic and chromatographic properties, in their resistance towards alkali, and in ultraviolet spectral absorption. The two components were composed of α and γ polypeptide chains, and the “fingerprints” of their tryptic digests followed
FETAL HEMOGLOBIN IN HIGH Hb-F ANOMALY

Fig. 5.—Schematic representation of the fingerprints of purified fetal hemoglobin of cord blood and of the alkali resistant hemoglobin component present in the blood of some members of the family P.

Hi = Histidine  Ar = Arginine  Ty = Tyrosine  Tr = Tryptophane

an identical pattern. It can, therefore, be assumed that in cases such as those described in this as well as other reports, an inherited persistence of true fetal hemoglobin occurs.

The genetic anomaly resulting in the persistence of fetal hemoglobin in adult life is difficult to understand. Herman and Conley\textsuperscript{10} stated that the abnormality appears to be genetically determined by a factor allelic with the genes responsible for the production of Hb-A, Hb-S and Hb-C. The hypothesis is consistent with their finding of a complete absence of normal Hb-A in cases heterozygous for Hb-S as well as for the genetic condition under discussion. In our family study, it is evident that the father (case II,2 with the hemoglobin pattern S-F) inherited the sickle-cell abnormality from his mother and the persistent high Hb-F anomaly from his father. From his marriage with a heterozygous Hb-S carrier, four children were born. One was homozygous Hb-S, one a heterozygous Hb-S carrier, one heterozygous for the persistent high Hb-F anomaly, and one child completely normal. The discovery of the normal child in this family should establish a heterozygosity for normal Hb-A in the father and, therefore, the non-allelism of the persistent high Hb-F anomaly and the genes responsible for the Hb-A, Hb-S and Hb-C. The analyses of different blood groups, however, have offered results, which makes it doubtful that case II,2 is the genetic parent of the normal case III,3. Our data are, therefore, not inconsistent with the hypothesis of Herman and Conley\textsuperscript{10} that the gene of the persistent high Hb-F anomaly, which is transmitted in a simple Mendelian man-
ner, appears to be allelic to the genes for Hb-A, Hb-S or Hb-C. The absence of normal Hb-A in the red blood cell hemolysate of the father (case II,2), confirmed both by starch-gel electrophoresis, and by CM-cellulose chromatography, shows that the presence of the persistent high Hb-F anomaly, when occurring together with a heterozygosity for Hb-S, prevents completely the production of normal Hb-A while the production of Hb-S is increased.

Our data from the 19 cases, in which the persistence of Hb-F occurred with Hb-A, support the findings of others\textsuperscript{10,20,28,31} that this genetic abnormality is clearly different from the thalassemia abnormality. The hematologic abnormalities characteristic for the thalassemia trait were absent, and the amounts of the Hb-A\textsubscript{2} fraction were slightly but significantly lower than in normals. It seems, therefore, that the anomalous gene is associated with a defect in the capacity for producing Hb-A\textsubscript{2}; a definite opinion, however, can only be reached by studying a homozygous carrier.

The results of our hematologic studies in the person (case II,2) with the FS combination also agree closely with those reported by other investigators.\textsuperscript{9,10,20,28,31} The case differs strikingly from cases homozygous for Hb-S as is shown in table 3 and table 4. The condition is also different from sickle-cell thalassemia disease.\textsuperscript{36} In this disease, the typical hematologic features of both inherited abnormalities are present. Normal adult hemoglobin is usually present in small amounts; the amount of fetal hemoglobin may rise to 30 per cent.

Of special interest are the observations made when the technic for the demonstration of fetal hemoglobin in the red blood cells\textsuperscript{24} is applied to the cases in which the persistent Hb-F gene is present in addition to either Hb-A or Hb-S. It was shown that all erythrocytes in these cases contained Hb-F in fairly constant amount. In sickle-cell disease, on the contrary, two cell-populations exist: a few cells contain large amounts of fetal hemoglobin while most cells are devoid of it.\textsuperscript{27} These observations support the hypothesis that the production of Hb-F in individuals with the double heterozygosity for Hb-S and the persistent high Hb-F anomaly is caused by a genetic disorder while the increased formation of Hb-F in sickle-cell disease may result indirectly from the severe anemia.

Also, it is likely that the relatively high per cent of Hb-F in each erythrocyte protects them from sickling and from destruction by hypotonic solutions or by lowering the pH of the environment. This conclusion is supported by observations of Allison,\textsuperscript{1} who demonstrated the inability of Hb-F, in contrast to Hb-A and Hb-C, to enter into the formation of molecular aggregates of Hb-S. The demonstration of two cell-populations in sickle-cell anemia also offers an explanation for the well-known fact that the severity of anemia is unrelated to the amount of fetal hemoglobin present.\textsuperscript{30} Since the fetal hemoglobin is not present in erythrocytes containing Hb-S, it is unable to protect them from destruction.

The distribution of Hb-F in the erythrocytes of a persistent high Hb-F carrier is also different from that found for cases with thalassemia major or thalassemia-Hb-E disease. In these thalassemia cases, two distinct cell-populations were observed: cells appearing as ghost forms and cells containing a high amount of Hb-F. This difference is consistent with the findings, that the genetic
basis for the synthesis of large quantities of Hb-F in the persistent high Hb-F anomaly is different from that involved in thalassemia. It has been suggested that thalassemia major is the result of a severe block in the synthesis of β-polypeptide chains (β-thalassemia), resulting secondarily in an increased synthesis of γ chains, and therefore of Hb-F. From our results may be concluded that this fetal hemoglobin is not equally distributed over all erythrocytes, suggesting the existence of hematopoietic sites in which Hb-F is synthesized predominantly. The equal distribution of Hb-F in the red blood cells of persistent high Hb-F carriers together with the absence of normal Hb-A in the FS combination and a normal synthesis of either Hb-A or Hb-S do not suggest a secondary accumulation of Hb-F as a result of a block in the synthesis of (altered) β-polypeptide chains. The complete absence on one chromosome of the gene regulating the synthesis of β-chains and the presence of an active gene regulating the production of γ-chains is the most likely genetic basis of the heterozygous persistent high Hb-F anomaly.

SUMMARY

Three generations of a Negro family having hereditary persistence of fetal hemoglobin in conjunction with the hemoglobins A and S were studied.

Genetic studies did not exclude the possibility that this anomaly is allelic with the genes responsible for the hemoglobins A, S, and C.

Structural investigations of the isolated abnormal hemoglobin fraction offered evidence that this component is identical with the fetal hemoglobin of the newborn child.

No striking clinical or hematologic abnormalities were found in members of the family who had high percentages of fetal hemoglobin in conjunction with either Hb-A or Hb-S. The per cent of Hb-A2 in the persistent high Hb-F carriers was found slightly below normal, while the total amount of circulating hemoglobins was increased. Previous reports of the complete absence of Hb-A in cases heterozygous for Hb-S as well as for the persistent high Hb-F anomaly have been confirmed by using refined technics available for hemoglobin analyses.

The fetal hemoglobin was distributed almost equally in all erythrocytes of individuals having this inherited anomaly. In this respect, the S-F abnormality differs from homozygous sickle-cell disease in which a mixed population of red blood cells, some with and most without Hb-F, has been demonstrated. It is suggested that the presence of some Hb-F in all erythrocytes of the individual with the S-F condition is the factor that protects the cells from sickling and from destruction.

The distribution of fetal hemoglobin in the red blood cells of persistent high Hb-F carriers was also different from that found in patients with thalassemia major and thalassemia-Hb-E disease. Two distinct cell populations, one with and the other without fetal hemoglobin, were found to be present in the blood of the thalassemia patients.

*A recent publication by Zuelzer et al. (Blood 7:393-408, 1961) refers to this family as an example of non-allelism between the persistent high Hb-F anomaly and the genes for Hb-A, Hb-S or Hb-C. The data presented above support allelism.
SUMMARIO IN INTERLINGUA

Esseva studiate tres generationes de un familia negre con persistencia hereditaria de hemoglobina fetal in conjunction con le presentia de hemoglobina A e hemoglobina S.

Studios genetic non excludeva le possibilitate que iste anomalia es alleclic con le genes responsabile pro le hemoglobinas A, S, e C.

Investigationes structural del isolate fraction anormal de hemoglobina produceva indicationes que iste componente es identic con le hemoglobina fetal del infante neonate.

Nulle frappante anormalitates clinic o hematologic esseva trovate in membros del familia qui habeva alte procentages de hemoglobina fetal in conjunction con hemoglobina A o hemoglobina S. In le portatores de persistencia de alte concentrationes de hemoglobina F, il esseva trovate que le procentage de hemoglobina A₂ esseva levemente infra le norma, durante que le quantitate total de hemoglobinas in le circulation esseva augmentate. Previe reportos del absentia complete de hemoglobina A in casos heterozygotic pro hemoglobin S o etiam pro persistencia de alte concentrationes de hemoglobina F ha essite confirmate per le uso del raffinate technicas nune disponibile pro analyses de hemoglobina.

Le hemoglobina fetal esseva distribuite quasi uniformemente in omne le erythrocytos del subjectos qui habeava iste anomalia hereditari. In iste respecto le anomalia S-F differiva ab homozygotic morbo de cellula falciforme in que un mixte population de erythrocytos ha essite demonstrate, incluse certes con e le majoritate sin hemoglobina F. Es presentate le these que possiblemente le presentia de un certe quantitate de hemoglobin F in omne le erythrocytos del subjecto con le condition S-F es le factor que protege le cellulas contra le falcification e contra le destruction.

Le distribution del hemoglobina fetal in le erythrocytos de portatores de persistencia de alte concentrationes de hemoglobina F differiva etiam ab le distribution trovate in patientes con thalassemia major e con thalassemia e morbo de hemoglobina E. Esseva trovate in le sanguine de patientes con thalassemia le presentia de duo distincte populationes cellular, i.e. un population con e le altere sin hemoglobina fetal.

ACKNOWLEDGMENTS

The authors are indebted to Miss Andrée Dozy and Mr. M. T. Bridges for capable technical assistance; to Dr. K. Betke of Freiburg, Germany, for introducing us to his technic; to the Department of Medicine, Medical College of Georgia and especially to Dr. J. Kemp for aiding us in the family study; to Dr. T. W. Middelbrooks of Union Point, Georgia, for his cooperation in helping to locate the family; to Dr. E. J. van Kampen, Groningen, The Netherlands, who made a copy of his study on the in vitro determination of Cr⁵¹ uptake by the red blood cells available to us prior to publication; to Dr. W. Shep- eard, Medical College of Georgia; to Dr. P. Levine and Mrs. M. Stroup, Ortho Research Foundation, Raritan, N. J., for testing the blood groups of some members of the family; to Drs. C. C. de Silva, Ceylon, and K. Pune, Utrecht, The Netherlands, who made blood samples of patients with thalassemia major and thalassemia-Hb-E disease available for this study; to Dr. L. D. Stoddard for his valuable help in preparing the manuscript and to Mr. B. Eng-
lrish and members of the Department of Medical Illustration of the Medical College of Georgia for preparing the figures.

REFERENCES


R. B. Thompson, M.D., Instructor of Pathology, Department of Pathology, Medical College of Georgia & Eugene Talmadge Memorial Hospital, Augusta, Ga.

J. W. Mitchener, M.D., Instructor of Pathology, Department of Pathology, Medical College of Georgia & Eugene Talmadge Memorial Hospital, Augusta, Ga.

Titus H. J. Huisman, Ph.D., D.Sc., Professor of Pathology & Biochemistry, Medical College of Georgia & Eugene Talmadge Memorial Hospital, Augusta, Ga.
Studies on the Fetal Hemoglobin in the Persistent High Hb-F Anomaly

R. B. THOMPSON, J. W. MITCHENER and TITUS H. J. HUISMAN

Updated information and services can be found at:
http://www.bloodjournal.org/content/18/3/267.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml