Hereditary Persistence of Fetal Hemoglobin: A Study of 79 Affected Persons in 15 Negro Families in Baltimore

By C. Lockard Conley, David J. Weatherall, Stuart N. Richardson, Marguerite K. Shepard and Samuel Charache

Hereditary persistence of fetal hemoglobin is a term tentatively applied to a specific inherited anomaly manifested throughout life by the presence of large amounts of fetal hemoglobin in the erythrocytes in the absence of anemia or clinical manifestations. The abnormality is attributable to a single autosomal genetic factor. Since the primary effect of the mutant gene is unknown, designations which identify the anomaly with thalassemia, or terms such as "the F gene," are undesirable. Persons heterozygous for the condition but otherwise normal have, on the average, 26 per cent fetal hemoglobin in their red cell hemolysates. This congenital abnormality must be differentiated from other disorders in which fetal hemoglobin may persist in adult life, in particular thalassemia, which at times it closely resembles.

Occurrence

First discovered in Nigeria by Edington and Lehmann,\textsuperscript{1,2} hereditary persistence of fetal hemoglobin has been encountered in Negroes in Uganda,\textsuperscript{3} Jamaica,\textsuperscript{4,5} and in the United States,\textsuperscript{6-12} and in a single Caucasian family in Greece.\textsuperscript{13} The only extensive survey to determine the frequency of the abnormality was performed in Baltimore, where five affected persons were found among 5000 Negroes examined.\textsuperscript{9,11} In a similar study in Philadelphia, one affected person was discovered in a survey of 1000 Negroes.\textsuperscript{8}

Characteristics of the Anomaly

The anomaly has been encountered in the homozygous and heterozygous state. Heterozygotes have displayed no other abnormality of hemoglobin synthesis (A-F) or have been heterozygous in addition for hemoglobin S (S-F), hemoglobin C (C-F) or thalassemia (Thal-F).

Homozygote: Only one person apparently homozygous for the anomaly has been discovered,\textsuperscript{11} a child of 33 months at the time of the present study. When first seen at the age of 15 months, he had hypochromic anemia which responded promptly and completely to the oral administration of ferrous
sulfate. Always in excellent health, he appears robust without palpable enlargement of the spleen or other physical abnormalities. Since the iron deficiency was corrected, microcytosis has persisted, other hematologic values have been normal (table 1), but marked abnormalities of the erythrocytes are present, with anisocytosis, poikilocytosis, target cells and occasional cells resembling spherocytes. There is no evidence of a hemolytic disorder. Only hemoglobin F is demonstrable in the red cell hemolysate, and no trace of hemoglobin A or A2 has been detected on repeated examinations. In the current study, the alkali-resistant fraction of hemoglobin has ranged between 87 and 92 per cent, but the method is known to yield erroneously low values for fetal hemoglobin at very high levels. Electrophoresis of hemoglobin on starch gel and on agar gel, and chromatography on Amberlite CG-50 columns, produced a single component with the characteristics of fetal hemoglobin. A fingerprint of a tryptic digest of the hemolysate showed only the peptides of fetal hemoglobin. Hemoglobin C added to the hemolysate could be detected by electrophoresis when it comprised only 0.1 per cent of the hemoglobin. Since hemoglobins C and A2 have the same mobility in this system, the hemolysate of the F homozygote apparently contains less than 0.1 per cent hemoglobin A2, and quite possibly none at all (fig. 1). In the absence of other demonstrable abnormalities, the morphologic aberrations of the red cells are presumed to be the result of the high concentration of fetal hemoglobin, which is perhaps organized within the cell in a manner different from that of normal hemoglobin.

**A-F heterozygotes:** Published reports contain reference to 110 persons so affected, including 64 from 15 families studied at The Johns Hopkins Hospital. Fifty were male and 60 female. Each carrier of the anomaly was ascertained during a survey or family study employing laboratory methods. All appeared to be in good health with neither anemia nor other clinical manifestations attributable to the abnormality. Comparison of hematocrit values and hemoglobin concentrations of affected persons with those of their normal (A-A) siblings revealed no appreciable differences. Target cells have been seen in the stained blood smears, but often in such small numbers that the blood was virtually indistinguishable from normal. Osmotic resistance of red cells was normal or slightly increased. Although the erythrocytes occasionally were described as slightly hypochromic in appearance, the mean corpuscular hemoglobin concentration usually was within the normal range, with a mean value of about 32 Gm. per 100 ml. Reticulocyte counts and the icterus index have been consistently normal, and the serum haptoglobin has been within normal limits in the few instances in which it was measured.

Alkali-resistant hemoglobin ranged between 17 and 33 per cent in the
Hereditary persistence of fetal hemoglobin

Fig. 1.—Absence of hemoglobin A₂ from the hemolysate of the F-homozygote. Electrophoresis was performed on vertical starch gel.² Hemoglobin A₂ appears as a heavy band in the hemolysate of normal adult blood but cannot be detected in that of the homozygote. Hemoglobin C is visible when added in a concentration of only 0.1 per cent of the total hemoglobin.

64 Hopkins cases and between 12 and 38 per cent in the 46 cases studied elsewhere. The alkali-denaturation method tends to yield incorrect values for fetal hemoglobin at very high and very low concentrations but is most accurate at the levels encountered in the A-F heterozygotes.¹⁴ The fetal fraction separated on agar gel at pH 6.0 was consistently slightly larger than the alkali-resistant fraction; in contrast, fetal hemoglobin separated by column chromatography was virtually identical in amount with the alkali-resistant fraction.⁹

Thirty-seven A-F heterozygotes from 10 families of the Hopkins group were available for examination in the present study. Ages ranged from 2 to 39 years. A carefully standardized and precise modification of the alkali-denaturation method was employed.¹⁶ All determinations were made in duplicate and results of replicate determinations differed by less than one per cent. Values for fetal hemoglobin ranged from 17.3 to 33.0 per cent with a mean of 25.95 per cent (fig. 2). No relation between age and the fetal fraction was demonstrated except that the highest values were encountered in children under 5 years (fig. 3). The lowest value also was observed in a child of 4 and was repeatedly confirmed over a period of 9 months. Twenty of the heterozygotes were re-examined after periods of 9 to 24 months. The mean difference between results obtained at these relatively long intervals was 3.0 per cent, and the largest difference encountered in any subject was 7.0 per cent. Thus
the variation in concentration of fetal hemoglobin in the group greatly exceeded that occurring in a single individual studied at different times. When the fetal hemoglobin fractions of the A-F heterozygotes are grouped in family units, there is a suggestion of interfamilial differences, although the range of variation is large within families (fig. 4). The cause of the obvious individual differences and of the suggested familial differences is unknown. The greatest divergence of family means occurs in groups studied by different investigators and may reflect methodologic discrepancies. Whether geographic or other factors play a role is not apparent. A possibility that has not been investigated is that the proportions of hemoglobins A and F vary with alterations in rate of erythropoiesis. The distribution of values within family units does not suggest polymorphism of the mutant gene responsible for persistence of fetal hemoglobin, but the possibility of other genetically determined modifying factors cannot be eliminated.

The distribution of hemoglobin F among the red cells is relatively uniform. All red cells contain about the same proportion of hemoglobin F
HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

Fig. 3.—Distribution of values for alkali-resistant hemoglobin of A-F heterozygotes in relation to age. The virtually horizontal regression line indicates that there is no tendency for the percentage of hemoglobin F to decline with increasing age.

Fig. 4.—Values for alkali-resistant hemoglobin of A-F heterozygotes grouped in family units. Results obtained in the present study are compared with those reported by other authors.

*This family contained 19 A-F members, but only the range and mean were reported.
Fig. 5.—Distribution of fetal hemoglobin in red cell populations. Blood films prepared by the method of Kleihauer and Betke\textsuperscript{16} were photographed and the optical density of the photographic images of 100 red cells measured as previously described.\textsuperscript{17} Normal adult blood was mixed with blood from the F-homozygote so that 26 per cent of the cells contained only hemoglobin F (A-A + F-F). In this mixture, the distribution of cell densities is completely bimodal with the dense F-F cells represented on the left. In contrast, the cell densities of the A-F heterozygote are narrowly distributed about a single intermediate mean, indicating that all cells contained similar proportions of hemoglobins A and F. The distribution of hemoglobin F in red cells of persons heterozygous for thalassemia, both of the high A\textsubscript{2} type (A-Thal) and the high F type (A-X), is strikingly heterogeneous but not bimodal (fig. 5), an apparently unique situation which has not been observed in any other condition in which increased levels of fetal hemoglobin occur.\textsuperscript{17}

Hemoglobin A\textsubscript{2} is significantly reduced in A-F heterozygotes when compared with values obtained in normal siblings (fig. 6). The range of values for the 37 persons in the present study was from 1.08 to 2.18 per cent (mean 1.80), whereas in 18 normal siblings the range was from 1.91 to 3.01 per cent (mean 2.5).

S-F heterozygotes: Twenty persons, including seven observed at The Johns Hopkins Hospital, are heterozygous for hereditary persistence of fetal hemoglobin and for hemoglobin S.\textsuperscript{1-7,12,18,19} Eleven are male and nine female. Ages range from 10 to 53 years. In general, these doubly affected persons have been in excellent health. Disproportionate elongation of the extremities, characteristic of sickle cell anemia, has not been described in this group and was not present in any of our cases. Abnormalities of blood flow, almost invariably detectable in the conjunctival vessels of patients with sickle cell anemia, were
absent in S-F heterozygotes examined in our clinic. In two instances mild joint pains have been described. Aseptic necrosis of the femoral head occurred in a reported case and in one of our own. Another of our patients has evidence of mild hemiparesis. Numerous pregnancies have been uncomplicated. The tip of the spleen was palpable in only two persons, one of whom had malaria at the time of the examination. One of our patients has mild nonhemolytic jaundice of unknown cause.

Anemia does not appear to be a manifestation of this condition, and there is no evidence of a hemolytic disorder. Of 18 cases in which measurements are recorded, the hemoglobin concentration exceeded 14 Gm. per 100 ml. in eight and in only four were values below 12 Gm. including one patient with terminal uremia. Hematologic data are summarized in table 2. Osmotic fragility of the red cells has been recorded as normal or decreased. Target cells and anisocytosis have usually been observed on the blood smear, and sickling of the erythrocytes was readily induced with sodium metabisulfite. Reticulocytes have been normal or only slightly increased. Serum haptoglobin was normal in the two cases of our group in which it was measured. Plasma
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Table 2.—Hematologic Values—F-Heterozygotes
Hereditary Persistence of Fetal Hemoglobin

Fig. 7.—Hemoglobin fractions separated on chromatographic columns (Amberlite CG-50). Normal umbilical cord blood contained hemoglobin A, eluted at 28°C, in addition to hemoglobin F, eluted at 4°C. In contrast, the hemolysate of the F-homozygote was eluted as a single component at 4°C. Hemoglobin A could not be detected in hemolysates of S-F and C-F heterozygotes but was readily recognized when added in amounts sufficient to comprise only 1.5 per cent of the total hemoglobin. Hemoglobin A was demonstrable in hemolysates of persons with S-thalassemia disease. The fractions separated by chromatography in this study were identified by electrophoretic methods.

Clearance of radioiron was normal in the one case studied. Blood from one of our S-F heterozygotes was injected into a normal volunteer after a portion of the red cells had been labeled with chromium. Survival of the transfused cells was estimated by measurement of radioactivity and also by identification of the S-F cells, employing the method of Kleihauer and Betke, which permits recognition of erythrocytes containing relatively high concentrations of fetal
hemoglobin. The chromium\textsuperscript{31} half-life was 25.5 days, and cells containing fetal hemoglobin were detected 90 days after injection; both procedures indicate that survival time of the S-F cells was almost normal.

Alkali-resistant hemoglobin ranged between 27.0 and 34.6 per cent in the seven Hopkins cases and between 15.0 and 35.0 per cent in those studied elsewhere (fig. 2). The case reported by Jacob and Raper\textsuperscript{3} with only 15 per cent fetal hemoglobin is exceptional because of the low value, the next lowest value being 22 per cent in another case described by the same authors. Hemoglobin A\textsubscript{2} is difficult to measure in hemolysates of the S-F heterozygotes because of the electrophoretic similarities of hemoglobins S and A\textsubscript{2}, but has been reported to be normal or slightly reduced.\textsuperscript{6,12} Sickle hemoglobin predominates, comprising on the average about 70 per cent of the total, and hemoglobin A has not been detected on careful study (fig. 7).

The asymptomatic S-F heterozygotes are of unusual clinical interest, because hemolysates of their blood are indistinguishable from those of some patients with severe sickle cell anemia. In rare instances of symptomatic sickle cell disease, fetal hemoglobin exceeds 25 per cent, and in one fatal case was 36 per cent.\textsuperscript{9} The striking clinical differences between the two conditions, in which the hemoglobin composition of the hemolysates may be identical, is explained by the difference in distribution of hemoglobin F among the red cells.\textsuperscript{7,9} In sickle cell anemia, hemoglobin F is heterogeneously distributed.\textsuperscript{9,17,22,23} Cells containing the lowest concentrations of fetal hemoglobin are rapidly destroyed, giving rise to hemolytic anemia; cells containing high concentrations of fetal hemoglobin tend to persist in the circulation.\textsuperscript{9,22} In the erythrocytes of the S-F heterozygotes, fetal hemoglobin is quite uniformly distributed; each red cell, containing a relatively high concentration of hemoglobin F, has an approximately normal life span.\textsuperscript{7,9} The absence of anemia has been attributed to the effect of fetal hemoglobin in preventing sickling of the erythrocytes under physiologic conditions.\textsuperscript{9} When S-F blood was deoxygenated, there were fewer sickled cells and less change in viscosity than in S-S blood treated in the same manner.\textsuperscript{18} In our studies the rate of change of viscosity of blood was measured during deoxygenation under controlled conditions. The blood of patients with sickle cell anemia showed a much more rapid increase in viscosity than that of the S-F heterozygotes. Blood from a patient with sickle cell anemia (S-S) and from the F homozygote (F-F) was mixed so that the hemolysate was similar in composition to that of the S-F heterozygotes. The striking difference in rate of increase in viscosity was again demonstrated, providing a demonstration in vitro of the important effect of distribution of fetal hemoglobin on the kinetics of sickling (fig. 8). The virtual absence of anemia in S-F heterozygotes provides evidence that the harmful effects of sickle hemoglobin are solely attributable to distortion of the red cells. If the sickling phenomenon does not occur, red cells have a normal life span even though they contain 70 per cent hemoglobin S. It seems likely that the concentration of hemoglobin F required to prevent sickling under physiologic conditions is little less than that occurring in the S-F individuals, for several of these persons have had clinical manifestations presumably attributable to intravascular sickling, including aseptic necrosis...
Fig. 8.—Rate of increase of viscosity of S-S and S-F blood during deoxygenation. Oxygenated blood, adjusted to a hematocrit value of 30 per cent, was progressively deoxygenated with a gas mixture containing 3 per cent oxygen, 5 per cent carbon dioxide and 92 per cent nitrogen at 37 C. under carefully controlled conditions of agitation and gas flow. Viscosity was estimated with a falling sphere viscometer, and is expressed as time required for a standard steel sphere to fall a fixed distance within a glass cylinder of uniform diameter. Oxygenated blood from an S-F heterozygote and from an S-S homozygote had the same viscosity. During deoxygenation, viscosity of the S-S blood rapidly increased, while that of the S-F heterozygote showed a much slower rate of increase. After 1 hour, the viscosity of the two specimens was similar. Dilution of S-S blood with normal red cells did not significantly decrease viscosity until almost half of the cells were of the non-sickling variety. In the experiment shown above, blood from an S-S homozygote was diluted with blood from the F-F homozygote so that the hemoglobin composition of the hemolysates of the two specimens was approximately the same.

of the femoral heads. Quite probably the S-F heterozygotes will be found to tolerate reduced oxygen tensions poorly, and like patients with sickle cell-hemoglobin C disease, will perhaps develop infarction of the spleen when exposed to moderately reduced atmospheric pressure. In view of these considerations, it is especially important to establish whether concentrations of fetal hemoglobin as low as 15 per cent are protective, as implied by Jacob and Raper.

C-F heterozygotes: Nine persons, including three studied at The Johns Hopkins Hospital, have been shown to be heterozygous for hemoglobin C and for hereditary persistence of fetal hemoglobin. Four are male and five female. Ages range from 9 to 70 years. These persons have had no symptoms attributable to the disorder, but the spleen was palpable in four cases. Blood smears show numerous target cells and some cells resembling spherocytes. Hematologic values are recorded in table 2. Hemoglobin concentrations ranged from 11.7 to 15.0 Gm. per 100 ml. Osmotic fragility of the red cells was nor-
mal in the four cases studied. In three cases there was a moderate increase in reticulocytes, and in one a slight increase in serum bilirubin. Serum haptoglobin was reduced in the one case of our group in which it was measured. Blood from a C-F heterozygote was injected into a normal volunteer after a portion had been labeled with chromium$^{51}$. Disappearance of radioactivity and of the cells containing fetal hemoglobin indicated a shortened survival time of the transfused erythrocytes; chromium$^{51}$ half-life was 15.5 days, and cells containing fetal hemoglobin could not be detected at 90 days although a few were present at 60 days.

The alkali-resistant hemoglobin ranged from 28.1 to 38.6 per cent (fig. 2). Fetal hemoglobin is fairly uniformly distributed among the red cells.$^{17}$ Hemoglobin A$_2$ has not been measured because it has the same electrophoretic mobility as hemoglobin C. Kraus and his associates$^{10}$ concluded that there was a small amount of hemoglobin A (2.6 and 4.5 per cent) in hemolysates of two of their four C-F heterozygotes, but the method employed was indirect and inadequate to establish this point. Sensitive electrophoretic and chromatographic technics failed to detect hemoglobin A in hemolysates from our C-F heterozygotes, and it can be said with certainty that if hemoglobin A was present, its concentration was less than one per cent (fig. 7).

The mild hemolytic disorder in the C-F heterozygotes is presumably attributable to the high concentration of hemoglobin C in the erythrocytes, but the mechanism by which this abnormal hemoglobin shortens the life span of the red cells is unknown.

**Thal-F heterozygotes:** Five persons in two Negro families have well-documented evidence of heterozygosity both for $\beta$ thalassemia and for hereditary persistence of fetal hemoglobin.$^{16,11}$ and four of these are in the Hopkins series. Fessas$^{13}$ has encountered a Caucasian family in which hereditary persistence of fetal hemoglobin appears to occur in one member with $\alpha$ thalassemia and in another with $\beta$ thalassemia. Of the doubly affected Negroes, four are male and one female, and ages range from 16 to 29 years. One has splenomegaly and has had episodes of discomfort in the left upper quadrant of the abdomen, but the others have been asymptomatic. Splenomegaly was also present in the Caucasian case in which $\beta$ thalassemia occurred. Hematologic data are summarized in table 2. There is little evidence of anemia, the hemoglobin concentrations ranging between 11.3 and 15.3 Gm. per 100 ml. Red cells consistently have shown increased resistance to osmotic lysis and pronounced morphologic abnormalities, including anisocytosis, poikilocytosis and target forms. The serum bilirubin was slightly elevated in two cases, and reticulocytes were modestly increased in four. Haptoglobin could not be detected in the serum of two of our heterozygotes but was normal in two similarly affected siblings. The half-life of the red cells, measured with chromium$^{51}$, was 20 days in the one person studied.$^{10}$

Alkali-resistant hemoglobin ranged from 66.8 to 70.7 per cent in the doubly affected Negroes, but was 43 per cent in the Greek person with $\beta$ thalassemia, and only 17.0 per cent in the person with $\alpha$ thalassemia. Fetal hemoglobin was quite uniformly distributed among the red cells in the Thal-F cases.
HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

studied in our laboratory. Hemoglobin A₂ was normal in all of the Negroes but was increased in the Caucasian case with ɤ thalassemia. These differences may reflect the well-known heterogeneity of the manifestations of thalassemia, and do not necessarily imply a racial factor.

The mild hemolytic disorder in the Thal-F heterozygotes is presumably attributable to thalassemia, since the homozygote for hereditary persistence of fetal hemoglobin displays no evidence of increased blood destruction.

Characteristics of the Fetal Hemoglobin

The alkali-resistant hemoglobin of persons with hereditary persistence of fetal hemoglobin has not been shown to differ from the fetal hemoglobin of umbilical cord blood. The kinetics of its reaction with alkali is similar. It cannot be differentiated by electrophoresis in various buffers and on several media including agar gel. It is eluted from chromatographic columns in a manner similar to that of fetal hemoglobin and displays the characteristic absorption curve in the ultraviolet spectrum. Fingerprints of tryptic digests are similar to those of fetal hemoglobin, and stains specific for certain amino acids do not show demonstrable differences. The amino acid composition is similar to that of fetal hemoglobin and isoleucine is present.

Many of these studies were repeated employing the hemolysate of the F homozygote. Mobility and configuration of the hemoglobin spots during electrophoresis on agar and starch gel were indistinguishable from those of fetal hemoglobin. The rate of alkali denaturation was identical to that of pure fetal hemoglobin obtained from cord blood by column chromatography. The absorption curve in the ultraviolet portion of the spectrum was superimposable on that of fetal hemoglobin. During chromatography on Amberlite CG-50 columns, employing developer 2 of Allen and associates, all of the hemoglobin was eluted in a single peak at 4 C, and none was eluted at 28 C. Fingerprints of tryptic digests of the hemolysate were identical with those of hemoglobin F, and specific stains produced the reactions typical of fetal hemoglobin.

Differentiation from Other Conditions in Which High Levels of Fetal Hemoglobin Occur

Concentrations of fetal hemoglobin as high as those encountered in the heterozygous state of hereditary persistence of fetal hemoglobin occur in several other conditions. The predominant hemoglobin of the fetus and newborn child is hemoglobin F, which has virtually disappeared by the age of 6 months. During neonatal life, the red cells of normal infants superficially resemble those of the adult heterozygotes, and the hemolysates may be indistinguishable when studied by alkali denaturation methods or by usual electrophoretic technics. In fact, however, there are pronounced differences. In the A-F heterozygotes, fetal hemoglobin is quite uniformly distributed among the red cells (fig. 5) in contrast to the very heterogeneous distribution in the red cells of the fetus and newborn. The red cell hemolysates of newborns are known to differ in certain non-heme proteins from the hemolysates of
Fig. 9.—Proteins stained with Buffalo black after electrophoresis of hemolysates on vertical starch gel at pH 8.5. Hemolysates 1 and 8 are from normal adult blood, 2 and 7 from the F-homozygote, and 3–6 from normal umbilical cord blood. Hemoglobins A and F form the large spots which advanced most rapidly. Hemoglobin A2 follows and is distinctly separate from the A and F components. The remaining bands are non-hemoglobin proteins, one of which is almost superimposed on the hemoglobin A2 band. Hemoglobin A2 is absent from the hemolysate of the F-homozygote, but the pattern of non-hemoglobin proteins is similar to that of normal adult blood and unlike that of the blood of the newborn infant. Some of the non-hemoglobin proteins have been identified as specific enzymes. This study indicates that, apart from the hemoglobin components, the red cells of the F-homozygote are comparable to those of the normal adult and unlike the erythrocytes of the newborn.

older persons. Examination of the non-heme proteins discernible by electrophoresis on starch gel shows that the hemolysate of the F homozygote has the adult pattern, strikingly different from the pattern obtained with normal cord blood (fig. 9). Furthermore, the oxygen dissociation curve of blood of an adult with F-thalassemia (69 per cent hemoglobin F) is identical with that obtained with blood of normal adults, and in contrast to the curve obtained with cord blood containing the same proportions of hemoglobins A and F.

High levels of fetal hemoglobin occur rarely in patients with certain acquired diseases. We have studied two cases of aplastic anemia and one of leukemia in which concentrations of hemoglobin F were of the order of those in A-F heterozygotes. In cases such as these, the co-existence of two conditions can be ruled out by appropriate family studies or more simply by a study
of the distribution of hemoglobin F in the red cells. In acquired diseases, the fetal hemoglobin is quite heterogeneously distributed.\textsuperscript{17}

More difficulty has been encountered in differentiating certain forms of thalassemia from hereditary persistence of fetal hemoglobin. Persons heterozygous for \(\beta\) thalassemia (high \(A_2\) type) often have slight elevations of levels of hemoglobin F, and in rare instances fetal hemoglobin reaches concentrations as high as those of the A-F heterozygotes.\textsuperscript{29-30} During the present study, 59 individuals with thalassemia minor were examined, including 36 Negroes, and 20 persons of Italian and 3 of Greek origin. Hemoglobin \(A_2\) ranged from 3.59 to 6.38 per cent (mean 4.98). Hemoglobin F varied between 0.38 and 6.60 per cent (mean 2.87). There was no correlation between levels of hemoglobins \(A_2\) and F in this group, nor were there significant differences between the hemoglobin fractions of the Negroes and Italians. In one exceptional family, however, thalassemia minor of the high \(A_2\) type was associated with high levels of fetal hemoglobin. In this family a 36 year old Negro woman had 4.2 per cent hemoglobin \(A_2\) and 17.9 per cent hemoglobin F, the remainder being hemoglobin A. Hematologic values were normal, and only an occasional target cell was seen on the stained smear. She might easily have been considered to represent an A-F heterozygote because of the high level of hemoglobin F and the virtual absence of hematologic abnormalities. However, the distribution of hemoglobin F in her red cells is strikingly heterogeneous, a pattern characteristically seen in thalassemia but not in hereditary persistence of fetal hemoglobin (fig. 5). Furthermore, the family study indicates that she does have thalassemia (a son has S-thalassemia disease).

A form of thalassemia has been described in persons of Mediterranean origin with normal values for hemoglobin \(A_2\) but with values for hemoglobin F ranging between 6 and 13 per cent.\textsuperscript{31} The blood picture is similar to that of thalassemia minor, and a child of a mating between an affected father and a mother with thalassemia minor of the high \(A_2\) type had thalassemia major. In a survey in Greece, 10 per cent of 110 instances of thalassemia minor were found to be of the high F type.\textsuperscript{32} In these cases hemoglobin \(A_2\) varied from 1.82 to 3.3 per cent (mean 2.6), and hemoglobin F from 3.5 to 20 per cent (mean 8.49). In every case the red cells appeared abnormal and in all but one, osmotic resistance was increased. We have studied a Negro family with “high F” thalassemia. The four heterozygotes studied have little or no anemia and reticulocytes are not increased. The red cells show microcytosis, hypochromia, anisocytosis, poikilocytosis and target forms. Resistance to osmotic lysis is increased. Hemoglobin \(A_2\) ranges from 1.68 to 2.04 per cent, and fetal hemoglobin from 5.3 to 13.9 per cent. Distribution of the fetal hemoglobin among the red cells is quite heterogeneous (fig. 5), a feature which distinguishes the condition from hereditary persistence of fetal hemoglobin.

In the light of present knowledge, it appears that some cases that have been referred to as examples of hereditary persistence of fetal hemoglobin actually were instances of other disorders. The persons with elevated values for hemoglobin F discovered in a survey in Africa by Neel and his associates\textsuperscript{33} had values well below those of F heterozygotes and probably had some form of thalassemia. In the report of Edington and Lehmann,\textsuperscript{8} J. A. S., in whom only
Table 3.—Offspring of F-Heterozygotes

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hemoglobins S and F were detected, had 5.2 per cent fetal hemoglobin, and a son had hemoglobin C-trait; accordingly, he could not have been an S-F heterozygote. The patient considered by McCormick and Humphreys34 to be a C-F heterozygote was severely anemic, and was reported to have 13.3 per cent hemoglobin A in the red cell hemolysate; family studies were not
adequate to establish a certain diagnosis. Similarly, the cases thought by Schneider and her associates\textsuperscript{25} to be S-F and C-F heterozygotes each had hemoglobin A in the red cell hemolysates and levels of fetal hemoglobin of only 13 per cent; presumably these were instances of S-thalassemia and C-thalassemia.

**Genetic Considerations**

The manner of inheritance of the fetal hemoglobin anomaly is well-established by available data. In every instance studied, an A-F heterozygote had one parent who was heterozygous for the condition. The sexes are about equally affected. Results of critical matings, listed in table 3, indicate that persistent fetal hemoglobin is transmitted by a single genetic factor apparently allelic with the genes for hemoglobin S and C and for \( \beta \) thalassemia, at the locus at which the structure of the \( \beta \) chain of globin is determined. That allelism rather than close linkage is involved is suggested by the absence of hemoglobin A in the S-F and C-F heterozygotes. Studies of both of the parents of 21 persons heterozygous for hereditary persistence of fetal hemoglobin and for a \( \beta \) chain abnormality are available (15 S-F, one C-F, 5 Thal-F). In every case, F was derived from one parent and the additional hemoglobin abnormality from the other. In a mating of S-F and A-S individuals, one child was found to have normal hemoglobin (A-A), but blood group studies suggested nonpaternity.\textsuperscript{12}

The fetal hemoglobin of the anomaly appears to be identical with that of umbilical cord blood, consisting of \( \alpha \) and \( \gamma \) chains (\( \alpha_2^A \gamma_2^F \)). The gene which determines the structure of the normal \( \gamma \) chain is not an allele of the \( \beta \) chain gene. The many differences in the structure of hemoglobins A and F are not likely to have arisen from a single mutation at the \( \beta \) chain locus in persons with the inherited anomaly. Accordingly, we have proposed that hereditary persistence of fetal hemoglobin is the consequence of a mutant gene, the primary effect of which is to prevent the synthesis of normal \( \beta \) chains. Because of the resultant deficiency of hemoglobin A, fetal hemoglobin is produced as a secondary and compensatory phenomenon.\textsuperscript{7} There is no limitation of the rate of hemoglobin synthesis so that anemia does not occur, even in the homozygous state. This fact establishes a fundamental difference between the anomaly and thalassemia. Furthermore, the anomaly is remarkably unvarying in contrast to thalassemia, which is much more heterogeneous.

If one assumes that the F heterozygotes have only one functional gene at the \( \beta \) chain locus, a gene-product relationship can be established. The A-F heterozygotes have, on the average, 26 per cent hemoglobin F, suggesting that one \( \beta^A \) gene can direct the synthesis of about 74 per cent of the hemoglobin. The S-F heterozygotes have higher levels of fetal hemoglobin, an observation in keeping with the concept that hemoglobin S is less readily made than hemoglobin A. If the values for hemoglobin F of the two S-F heterozygotes reported by Jacob and Raper\textsuperscript{3} are eliminated, the mean becomes 28.9, which is significantly different from that of the A-F group. The mean value for fetal hemoglobin in the C-F heterozygotes is 33.9 per cent, suggesting that hemoglobin C is synthesized less readily than hemoglobin S. And the very
high values for hemoglobin F in the F-thalassemia heterozygotes reflects the impotence of the thalassemia gene in directing the synthesis of hemoglobin A.

The low values for hemoglobin A₂ in A-F heterozygotes and its absence in the F homozygote has complicated the interpretation of the anomaly. Since the structure of the δ chain of hemoglobin A₂ is determined at a locus distinct from that of the β chain of hemoglobin A, the anomaly appears to involve suppression of activity of two nonallelic genes. A suggested explanation is that the mutation affects an "operator gene" controlling the function of two structural loci that presumably are closely linked.³⁄₇⁸⁰ If the function of the δ chain locus is wholly suppressed by the mutant gene causing hereditary persistence of fetal hemoglobin, A-F heterozygotes, in the absence of an α chain abnormality, invariably should have only one A₂ component and should be incapable of having a "split" A₂. Mutation of the δ chain gene is known to occur, producing in heterozygotes two types of hemoglobin A₂ (A₂ and B₂) separable by electrophoresis.³⁷ Kraus and his associates¹⁰ encountered an A-F heterozygote who was reported to have both A₂ and B₂ fractions. However, Kraus states in a personal communication that restudy of his A-F person shows that only hemoglobin B₂ is present, and that A₂ cannot be detected. Accordingly, the observation gives strong support to the hypothesis that the function of a linked δ chain gene is completely suppressed by the gene for the F anomaly. There are other conditions in which a reciprocal relationship is observed between hemoglobins A₂ and F.¹⁵ One of our adult patients with acquired aplastic anemia had an elevated fetal hemoglobin (20.6 per cent) and a low value for hemoglobin A₂ (0.9 per cent). In this instance the relationship may be nongenetic in origin.

The red cells of the fetus contain high concentrations of fetal hemoglobin and little or no hemoglobin A₂. However, there is no justification in considering that the anomaly represents persistence into adult life of the condition which exists in the fetus. The remarkably uniform distribution of hemoglobin F regularly seen in the erythrocytes in the inherited condition is not encountered at any stage of fetal life.¹⁷ The red cells of persons with the anomaly appear similar to those of normal adults apart from their hemoglobin components; they resemble adult red cells in the type of non-heme proteins demonstrable by electrophoresis, and the oxygen dissociation curve is adult in type even when the fetal hemoglobin content is 70 per cent.

Summary

Hereditary persistence of fetal hemoglobin is an anomaly of hemoglobin production apparently caused by a mutant gene that inhibits synthesis of hemoglobins A and A₂. Alkali-resistant hemoglobin indistinguishable from hemoglobin F of umbilical cord blood is produced, presumably as a compensatory phenomenon, so that neither anemia nor hypochromia of the red cells occurs. The data summarized are compatible with the hypothesis that function of the loci of the β and δ chains of globin is wholly suppressed, quite possibly by a mutant "operator" gene affecting linked structural loci. Heterozygotes for the anomaly have high concentrations of hemoglobin F in the erythrocytes,
with a remarkably uniform distribution of fetal hemoglobin throughout the red cell population. Erythrocytes of persons with the anomaly resemble adult red cells with respect to the non-hemoglobin proteins and the oxygen dissociation curve. Experience with 79 affected Negroes in Baltimore is compared with that reported by other investigators. The occasional difficulty in differentiating the anomaly from other conditions, particularly thalassemia, is emphasized.

**Summario in Interlingua**

Persistentia hereditari de hemoglobina fetal es un anormalitate in le production de hemoglobina que es apparentemente causate per un mutante gen que inhibi le synthese del hemoglobinas A e A₂. Hemoglobina alcali-resistente que non es distinguibile ab le hemoglobina F del cordon umbilical es producite presumitemente como un phenomeno compensatori, de manera que il occurre ni anemia ni hypochromia del erythrocytos. Le datos summarisate es in harmonia con le hypothese que le function del locos del catenas β e δ de globina es completemente supprimite, possibilissimemente per un mutante gen "de operation" que affice interligate locos structural. Heterozygoticos pro iste anormalitate ha alte concentrationes de hemoglobina F in le erythrocytos, con un remarcable uniformitate del distribution de hemoglobina fetal a transverso le population erythrocytic. Le erythrocytos de personas con iste anormalitate resimila adulte erythrocytos con respecto a lor proteinas non-hemoglobinice e al curva de dissociation de oxygeno. Le experientias con 79 afficite negros in Baltimore es comparate con le datos reportate per altere investigatores. Es sublineate le dificultate que on incontra occasionalmente in le differentiation inter iste anormalitate e altere conditiones, como per exemplo thalassemia.

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