Hemoglobin F<sub>Houston</sub>: A Fetal Variant

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Many genetically determined variants of the β polypeptide chain of adult hemoglobin A are known, but only two proved variants of the corresponding γ chain of fetal hemoglobin F have been described. The present report deals with another γ chain variant, found in the cord blood sample of a healthy, term Negro infant (R. Wo.). The variant is designated hemoglobin F<sub>Houston</sub>, since the propositus lives in that city.

Methods

Erythrocytes were washed three times with saline and hemolyzed with water and toluene. The clear hemoglobin layer was collected after centrifugation and analyzed by the following methods:

1. Paper electrophoresis, barbital buffer, pH 8.6, ionic strength 0.05. Paper (and gel) electrophoregrams were stained with benzidine.

2. Starch gel electrophoresis in a buffer of pH 8.6 containing 0.05 M tris(hydroxymethyl)amino methane, 0.002 M ethylene-diamine-tetracetic acid (EDTA), and 0.015 M boric acid (TEB buffer). A potential gradient of 8–10 V/cm. was applied to the horizontal gel for about 18 hrs.


5. Chromatography, Amberlite resin, IRC-50, diethylaminoethyl cellulose (DEAE), carboxymethyl cellulose (CMC), and CM Sephadex C-50.

6. Alkali denaturation.

The variant hemoglobin was isolated from the blood samples of R. Wo. by DEAE chromatography, after prior separation by starch grain electrophoresis, barbital buffer, pH 8.6. The isolated variant was tested for homogeneity by starch gel electrophoresis and examined by the following methods:

1. Ultraviolet (UV) absorption spectrum of CO hemoglobin in phosphate buffer, pH 8.0, in the Beckman D.U. spectrophotometer.

2. Immunodiffusion with rabbit antiserums, by modifications of previous methods.

Hemoglobin antigens for injection of rabbits and for immunodiffusion were prepared chromatographically (DEAE or CM Sephadex C-50, or both) after prior isolation by starch grain electrophoresis, and then tested for homogeneity by starch gel electrophoresis before use. Immunodiffusion was allowed to proceed for one week, after which the plates were washed for at least 24 hours with saline and water, and then stained with benzidine.

3. Hybridization, by the method of Itano and Singer.

4. Tryptic peptide patterns, by the procedure of Jones.

5. After hydrolysis of the variant with 6N HCl at 110 C. for 22 hours and 70 hours.
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Fig. 1.—Paper electrophoresis, barbital buffer, pH 8.6, of hemolysates of R. Wo. and parents.

Fig. 2.—Starch gel electrophoresis, TEB buffer, pH 8.6, of hemolysate of R. Wo., compared with others. (a) Cord blood, and (b) sample at 4 months of age.

respectively, amino acid analyses were made on a Beckman/Spinco amino acid analyzer, modified with long path flow cells, as described by Jones and Weiss. 15

RESULTS AND DISCUSSION

In zone electrophoresis in alkaline buffers, hemoglobin F<sub>Houston</sub> moves more slowly toward the anode than does hemoglobin S, the difference between the two being especially marked in starch gel electrophoresis (Figs. 1 and 2). In citrate agar electrophoresis, pH 6.2, hemoglobin F<sub>Houston</sub> does not separate
from hemoglobin F. In chromatography on Amberlite resin, IRC-50, the variant moves between hemoglobins F and A. In chromatography on columns of CMC and CM Sephadex C-50, the variant does not separate clearly from hemoglobin F; in chromatography on DEAE columns, it is eluted immediately after hemoglobin A₂. When estimated quantitatively in DEAE chromatography, hemoglobin F₁₁₁₁₁ constituted about 15 per cent of the total cord blood hemoglobin of R. Wo. Alkali denaturation tests were not performed on R. Wo's cord blood sample, but in blood samples drawn when he was 7 weeks and 4 months old, the alkali denaturation values were 50 per cent and 4.5 per cent, respectively.

In the blood sample obtained from R. Wo. at 7 weeks of age, the amount of variant appeared slightly less than in the cord blood. When the infant was last seen, at 4 months of age, both hemoglobin F₁₁₁₁₁ and normal hemoglobin F were barely demonstrable in the hemolysate, the remainder of the hemoglobin being of the normal adult type (Fig. 2b).

The hemolysates of R. Wo.'s parents and two siblings resolved into the normal adult pattern in zone electrophoresis (Fig. 1). However, when the hemolysates of the parents were examined in chromatography on Amberlite resin IRC-50, a trace amount of a fraction moving like hemoglobin F₁₁₁₁₁ (and only a very faint trace of normal hemoglobin F) was consistently demonstrable in the sample from the father. The mother's sample resolved into the normal adult pattern. The father's hemolysate (containing less than 1 per cent hemoglobin F by the method of alkali denaturation) was subjected to starch grain electrophoresis, barbital buffer, pH 8.6. That portion of the starch containing hemoglobin A₂ was excised widely toward the anode, and the hemoglobin was eluted from it. The eluted hemoglobin—about 3.5 per cent of the total—was concentrated by dialysis against polyethylene glycol, after which it was examined in starch gel electrophoresis, TEB buffer, pH 8.6. The resultant pattern was that of hemoglobin A₂, with a small contamination of hemoglobins F and A, and with another small fraction migrating like hemoglobin F₁₁₁₁₁.

In the UV absorption spectrum of the variant isolated from the cord blood sample of R. Wo., the characteristic "fetal type" of tryptophan fine structure band appeared, at about 288–289 mpμ.

Immunodiffusion tests were performed with rabbit antiserums which specifically differentiated α, β, γ, and δ polypeptide chains but did not distinguish small amino acid differences within these chains. In tests such as that with F antiserum in Figure 3, hemoglobin F₁₁₁₁₁ appears identical with hemoglobin F, but different from those hemoglobins which lack either α or γ polypeptide chains (hemoglobins Bart's, A, S, C, and A₂). Similar results were obtained with antiserums which were appropriately absorbed so that each contained antibody against only one type of polypeptide chain. Hemoglobins F and F₁₁₁₁₁ reacted with antibody against α or γ chains, and both failed to react with antibody against β or δ chains.

When hemoglobin F₁₁₁₁₁ was hybridized with the β chain variant, hemoglobin C, no new products were formed (Fig. 4). However, when hemoglobin F₁₁₁₁₁ was hybridized with the fetal form of the α chain variant, Gₐ₈₉₅₅₂, two new products appeared (Fig. 4). Of these, one moved like hemoglobin F
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1. Cord blood of R. Wo.
2. Mixture of hemoglobins F\textsubscript{Hou} and F\textsubscript{GPhila}, Control
3. Same as 2, hybridized
4. AS, Control
5. Mixture of hemoglobins F\textsubscript{Hou} and C, Control
6. Same as 5, hybridized
7. Hemolysate of infant with hemoglobin C trait, Control

Fig. 4.—Hybridization of hemoglobin F\textsubscript{Houston} with hemoglobin F\textsubscript{GPhila} and hemoglobin F\textsubscript{Houston} with hemoglobin C. Starch gel electrophoresis, TEB buffer, pH 8.6.

\((\alpha_2\gamma_2)\). The mobility of the other was that to be expected of the doubly abnormal hybrid molecule, \(\alpha_2\gamma_2\gamma'_2\text{F}_{\text{Houston}}\).

Peptide chromatograms of hemoglobin F\textsubscript{Houston} revealed the presence of both \(\alpha\) and \(\gamma\) polypeptide chains but failed to disclose an abnormality. Insufficient material was available for further peptide analyses. Data on the amino acid analyses are presented in Table 1. They indicate that in hemoglobin F\textsubscript{Houston} one amino acid (alanine) is increased in ratio by about 1 to 2 residues per mole, while two others (serine and glutamic acid) are similarly decreased. All other amino acids are present in ratios similar to those of hemoglobin F. The low serine value in hemoglobin F\textsubscript{Houston} is of doubtful significance, because, as is well known, serine (and threonine) is partially destroyed during hydrolysis, so that the possibility of inaccurate estimation cannot be excluded. The low value
### Table I. Amino Acid Composition of Hemoglobin F<sub>Houston</sub> in Residues per Mole

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Hb F&lt;sub&gt;Houston&lt;/sub&gt;</th>
<th>Normal Hb F (Theoretical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>46.0</td>
<td>46</td>
</tr>
<tr>
<td>His</td>
<td>34.0</td>
<td>34</td>
</tr>
<tr>
<td>Arg</td>
<td>11.7</td>
<td>12</td>
</tr>
<tr>
<td>Asp</td>
<td>50.6</td>
<td>50</td>
</tr>
<tr>
<td>Thr</td>
<td>37.5</td>
<td>38</td>
</tr>
<tr>
<td>Ser</td>
<td>42.9</td>
<td>44</td>
</tr>
<tr>
<td>Glu</td>
<td>32.7</td>
<td>34</td>
</tr>
<tr>
<td>Pro</td>
<td>21.7</td>
<td>22</td>
</tr>
<tr>
<td>Gly</td>
<td>39.7</td>
<td>40</td>
</tr>
<tr>
<td>Ala</td>
<td>66.3</td>
<td>64</td>
</tr>
<tr>
<td>Cys/2</td>
<td>4.0</td>
<td>4</td>
</tr>
<tr>
<td>Val</td>
<td>52.6</td>
<td>52</td>
</tr>
<tr>
<td>Met</td>
<td>7.9</td>
<td>8</td>
</tr>
<tr>
<td>Isoleu</td>
<td>8.0</td>
<td>8</td>
</tr>
<tr>
<td>Leu</td>
<td>70.1</td>
<td>70</td>
</tr>
<tr>
<td>Tyr</td>
<td>10.0</td>
<td>10</td>
</tr>
<tr>
<td>Phe</td>
<td>30.0</td>
<td>30</td>
</tr>
<tr>
<td>Tryp</td>
<td>†</td>
<td>8</td>
</tr>
</tbody>
</table>

*Best estimates from a 22 hr. and 70 hr. hydrolysis.
†Not measured.

for glutamic acid and the high value for alanine appear to be significant. Probably the most plausible explanation of our findings is that there is a substitution of an alanyl for a glutamyl residue in the \( \gamma \) chain of hemoglobin F<sub>Houston</sub>. Such a substitution, glu → ala, is known to occur at two different sites in the \( \beta \) chain of two variants of hemoglobin A—hemoglobins G<sub>HbLyon</sub><sup>17</sup> and G<sub>Connshatta</sub><sup>18</sup> respectively. This substitution in hemoglobin F<sub>Houston</sub> would be consistent with the electrophoretic data, since, in zone electrophoresis in alkaline buffers, hemoglobin F<sub>Houston</sub> is slower than hemoglobin F by about as much as hemoglobin G is slower than hemoglobin A.

The presence of a trace of hemoglobin F<sub>Houston</sub> in the hemolysate of the father of the propositus suggests that the genetic transmission was through this parent, and that the variant gene, like the gene for normal hemoglobin F, is functional at a low level throughout life. We have made a similar observation for the \( \gamma \) chain variant, hemoglobin F<sub>Texas</sub><sup>2</sup> found in the cord blood samples of three siblings and several of their paternal cousins. Recently, we have found a trace amount of hemoglobin F<sub>Texas</sub> in the hemolysate of the father of these siblings, as well as in two of their cousins whose cord blood samples contained hemoglobin F<sub>Texas</sub>, but whose hemoglobin patterns are now of the normal adult type.

**Note added in proof:** Huisman et al.<sup>19</sup> have recently described a gamma chain hemoglobin variant, F<sub>Warren</sub>, whose properties are similar or identical to those of hemoglobin F<sub>Houston</sub>.

### Summary

A fetal hemoglobin variant, designated hemoglobin F<sub>Houston</sub>, was found in the cord blood sample of a healthy, term Negro infant. The variant, compris-
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ing about 15 per cent of the total cord blood hemoglobin, diminished concomitantly with hemoglobin F, and it was barely detectable in the blood when the infant was 4 months old. The hemolysates of the parents and two siblings resolved into the usual adult pattern, but a trace amount of a fraction similar to hemoglobin F₄₅₀ was present in the father’s hemolysates and not in the mother’s.

The ultraviolet absorption spectrum indicates that hemoglobin F₄₅₀ contains γ polypeptide chains, and immunologic studies reveal the presence of both α and γ chains. In hybridization tests the alteration appears in the γ chain.

Peptide chromatograms of hemoglobin F₄₅₀ indicated the presence of α and γ chains, but failed to reveal an abnormality. Amino acid analyses suggest that there may be a substitution of an alanyl for a glutamyl residue.

SUMMARIO IN INTERLINGUA

Un variante fetal de hemoglobina, designate como hemoglobina F₄₅₀, esseva trovate in un specimen de sanguine del corda umbilical de un normal infante negre nascite a termino. Le variante, representate circa 15 pro cento del total hemoglobina del sanguine del corda umbilical, declinava concomitantemente con hemoglobina F, e illo esseva a pena detegibile in le sanguine quando le infante habeva attingite le etate de 4 menses. Le hemolysatos del parentes e de duo fraternos se resolveva ad in le usual configurationes adulte, sed un quantitate-tracia de un fraction simile a F₄₅₀ esseva presente in le hemolysatos del patre, ben que non in illos del matre.

Le spectro de absorption ultraviolette indica que hemoglobina F₄₅₀ contine catenas polypeptidic γ, e studios immunologic revela le presentia de catenas α e etiam γ. In tests de hybridisation, le alteration appare in le catena γ.

Chromatogrammas peptidic de hemoglobina F₄₅₀ indica le presentia de catenas α e γ sed non revelava ulle anormalitate. Analyses de amino-acido suggestiona que il se tracta possibilemente de un reemplacimento de un residuo glutamylic per un alanylic.

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REFERENCES


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