Hemoglobin $F_{\text{Houston}}$: A Fetal Variant

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Many genetically determined variants of the $\beta$ polypeptide chain of adult hemoglobin A are known, but only two proved variants of the corresponding $\gamma$ chain of fetal hemoglobin F have been described. The present report deals with another $\gamma$ chain variant, found in the cord blood sample of a healthy, term Negro infant (R. Wo.). The variant is designated hemoglobin $F_{\text{Houston}}$, 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. Immuno-diffusion with rabbit antiseraums, 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 they were 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.
HEMOGLOBIN $F_{Houston}$: A FETAL VARIANT

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.\textsuperscript{15}

Results and Discussion

In zone electrophoresis in alkaline buffers, hemoglobin $F_{Houston}$ 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 $F_{Houston}$ 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 $F_{\text{Houston}}$ 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 $F_{\text{Houston}}$ 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 $F_{\text{Houston}}$ (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 $F_{\text{Houston}}$.

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 bands appeared, at about 288-289 mp.

Immunodiffusion tests were performed with rabbit antiserums which specifically differentiated $\alpha$, $\beta$, $\gamma$, and $\delta$ 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 $F_{\text{Houston}}$ appears identical with hemoglobin F, but different from those hemoglobins which lack either $\alpha$ or $\gamma$ 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 $F_{\text{Houston}}$ reacted with antibody against $\alpha$ or $\gamma$ chains, and both failed to react with antibody against $\beta$ or $\delta$ chains.

When hemoglobin $F_{\text{Houston}}$ was hybridized with the $\beta$ chain variant, hemoglobin C, no new products were formed (Fig. 4). However, when hemoglobin $F_{\text{Houston}}$ was hybridized with the fetal form of the $\alpha$ chain variant, $G_{\text{Philadelphia}}^{16}$, two new products appeared (Fig. 4). Of these, one moved like hemoglobin F
HEMOGLOBIN F\textsubscript{Houston}: A FETAL VARIANT

**Fig. 3.**—Immunodiffusion reactions of hemoglobin F\textsubscript{Houston} compared with other types of hemoglobin. F antiseraum in center trough, various hemoglobins in surrounding wells, as indicated. Stained with benzidine.

1. Cord blood of R. Wo.
2. Mixture of hemoglobins F\textsubscript{Houston} and F\textsubscript{Philadelphia}, Control
3. Same as 2, hybridized
4. AS, Control
5. Mixture of hemoglobins F\textsubscript{Houston} 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{Philadelphia} and hemoglobin F\textsubscript{Houston} with hemoglobin C. Starch gel electrophoresis, TEB buffer, pH 8.6.

\((\alpha\gamma_2)\). The mobility of the other was that to be expected of the doubly abnormal hybrid molecule, \(\alpha\gamma_2\text{GPhiladelphia}\).

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 1—Amino Acid Composition of Hemoglobin F_{Houston} in Residues per Mole

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Hb F_{Houston}</th>
<th>Normal Hb F (Theoretical)</th>
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</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>
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<td>64</td>
</tr>
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<td>Cys/2</td>
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</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 γ chain of hemoglobin F_{Houston}. Such a substitution, glu → ala, is known to occur at two different sites in the β chain of two variants of hemoglobin A—hemoglobins G_{Habens}^{17} and G_{Habens}^{18} respectively. This substitution in hemoglobin F_{Houston} would be consistent with the electrophoretic data, since, in zone electrophoresis in alkaline buffers, hemoglobin F_{Houston} 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_{Houston} 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 γ chain variant, hemoglobin F_{Tex}, 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_{Tex} in the hemolysate of the father of these siblings, as well as in two of their cousins whose cord blood samples contained hemoglobin F_{Tex}, but whose hemoglobin patterns are now of the normal adult type.

Note added in proof: Huisman et al. have recently described a gamma chain hemoglobin variant, F_{Warron}, whose properties are similar or identical to those of hemoglobin F_{Houston}.

SUMMARY

A fetal hemoglobin variant, designated hemoglobin F_{Houston}, was found in the cord blood sample of a healthy, term Negro infant. The variant, compris-
HEMOGLOBIN F Houston: A FETAL VARIANT

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 F Houston was present in the father’s hemolysates and not in the mother’s.

The ultraviolet absorption spectrum indicates that hemoglobin F Houston 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 F Houston 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 F Houston, esseva trovate in un specimen de sanguine del corda umbilical de un normal infante negro 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 F Houston esseva presente in le hemolysatos del patre, ben que non in illos del matre.

Le spectro de absorption ultraviolette indica que hemoglobina F Houston 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 F Houston 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 reemplaciamento de un residuo glutamyclic per un alanylic.

ACKNOWLEDGMENTS

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REFERENCES


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