A New Variant of Human Fetal Hemoglobin: Hb F\textsubscript{Roma}

By E. Silvestroni and I. Bianco

During the past two years in our laboratory, the hemoglobin patterns of umbilical cord blood of newborns have been investigated.\textsuperscript{1-3} In one cord blood, an abnormal hemoglobin fraction has been identified. This hemoglobin has been called Hb F\textsubscript{Roma}. On the basis of its electrophoretic mobility at pH 8.6, this fraction was at first identified as hemoglobin Bart's. Later, however, after more careful investigation, this hemoglobin has shown an electrophoretic mobility at pH 6 and a behavior in hybridization tests which differentiates it, beyond any doubt, from hemoglobin Bart's.

The umbilical cord blood from which the abnormal hemoglobin was first identified was studied by various methods. The alkali-resistant hemoglobin was determined by the technic of Singer et al.\textsuperscript{4} The abnormal hemoglobin (Hb F\textsubscript{Roma}) was separated from starch block electrophoresis by the methods of Kunkel and Wallenius.\textsuperscript{5}

Agar gel electrophoresis was performed according to the method of Robinson et al.\textsuperscript{6} Amberlite IRC-50 chromatography was done utilizing the technic of Huisman and Prins.\textsuperscript{7}

Hybridization tests\textsuperscript{8,9} were performed using hemolysates containing hemoglobins with known alterations in specific peptide chains.

Other hematologic studies were done utilizing standard hospital technics.

Case Report

A healthy, full-term, baby girl (C. S.) was born to apparently healthy parents in a hospital in Rome. The father was born in the province of Frosinone and the mother was a native of Calabria. Examination of the cord blood revealed the presence of the above abnormal hemoglobin.

The abnormal hemoglobin, which we have provisionally named Hb F\textsubscript{Roma}, showed on paper electrophoresis at pH 8.6 a mobility greater than that of hemoglobin N and slightly less than that of hemoglobin H (fig. 1). On paper electrophoresis at pH 6, Hb F\textsubscript{Roma} was slower than hemoglobin F but faster than hemoglobin Bart's (fig. 2). In the hemoglobin \textit{in toto} of the umbilical cord blood, the alkali-resistant hemoglobin was 79 per cent and the Hb F\textsubscript{Roma} was 17 per cent.

Other examinations conducted during the first 5 months of life revealed a gradual decrease of both hemoglobin F and Hb F\textsubscript{Roma} (table 1). During the 5th month of life, hemoglobin F was 3.5 per cent, Hb F\textsubscript{Roma} had completely disappeared, and hemoglobin A\textsubscript{2} was 1.9 per cent. At the time of writing the baby was developing normally and showed a normal hematologic picture.

Examination of the parents' blood revealed no abnormal hemoglobins and Hb A\textsubscript{2} and Hb F fractions were normal (table 1). Also, the hematologic picture was normal in the parents, excluding the presence of a mild hypochromic anemia in the mother during the 1st month postpartum. Blood group determinations revealed no incompatibility between the parents and their daughter.

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Fig. 1.—Paper electrophoresis of cord blood hemolysate containing Hb F_{Roma} (above), of Hb A-H (center) and Hb A-N (below). Glycine buffer pH 8.6, ionic strength 0.04, staining with bromphenol blue. Mobility of fraction F_{Roma} is greater than that of Hb N, slightly less than that of Hb H.

Fig. 2.—Paper electrophoresis of cord blood hemolysate containing Hb F_{Roma} (above) and cord blood hemolysate containing Hb Bart's (below). Phosphate buffer pH 6, ionic strength 0.04. Hb F_{Roma} is markedly slower than Hb F but faster than Hb Bart's.

Study of Hb F_{Roma}

On agar gel electrophoresis at pH 6, Hb F_{Roma} is identical to and does not separate from Hb F (fig. 3). On Amberlite IRC-50 chromatography at pH 6.2, this fraction shows a mobility which is markedly greater than that of the remaining Hb F (fig. 4). In the U. V. it has an absorption spectrum similar to that of Hb F, with the point of greatest inflexion of the tryptophan band at the same wave length as Hb F, and with a very slight increase after the point of
maximum inflexion, as noted in Hb Bart's also (fig. 5). In the total hemolysate containing Hb F\textsubscript{Roma}, if the alkali-resistance test of Singer et al. is performed, Hb F\textsubscript{Roma} remains unaffected by exposure to alkali (fig. 6).

Experiments of hybridization of Hb F\textsubscript{Roma} have been conducted using hemoglobins which have known alterations in either the alpha or beta chain. The purpose of these experiments was to ascertain whether Hb F\textsubscript{Roma} is composed of one or two different types of chains, and in the latter case, in which of these two types the specific structural alteration is located. The experiments were performed by the method of Gammack et al.\textsuperscript{8} which is a microscale adaptation of that described by Itano and Robinson.\textsuperscript{9}

Hybridization of Hb F\textsubscript{Roma} with Hb S, which has a known alteration in the beta chain (\(\alpha^4\beta^8\))\textsuperscript{10} produced only the hemoglobin fractions present in the control hemolysates (fig. 7). On the contrary, hybridization with Hb L\textsubscript{Ferrara}, which is an Hb altered in the alpha chain (\(\alpha^1\beta^4\))\textsuperscript{11} gave origin (fig. 7) to a new fraction intermediate between Hb F\textsubscript{Roma} and Hb L\textsubscript{Ferrara}, with a mobility identical to that of Hb A\textsubscript{1} (fig. 8). Hybridization of Hb F\textsubscript{Roma} with Hb

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**Table 1.** Hematologic and Hemoglobinic Data of the Newborn Carrier of Hb F\textsubscript{Roma} and of the Parents

<table>
<thead>
<tr>
<th>Laboratory Findings</th>
<th>Case of Hb F\textsubscript{Roma}</th>
<th>Father</th>
<th>Mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (Gm./100 ml.)</td>
<td>At birth</td>
<td>45 days</td>
<td>3 months</td>
</tr>
<tr>
<td>RBC 10(^3)/mm.(^3)</td>
<td>11</td>
<td>13.5</td>
<td>12</td>
</tr>
<tr>
<td>Erythrocytic morphology</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Osmotic fragility</td>
<td>0.32-0.48</td>
<td>0.32-0.46</td>
<td></td>
</tr>
<tr>
<td>MCV (\mu)</td>
<td>100</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>Hb F\textsubscript{Roma} in % of total Hb</td>
<td>17</td>
<td>12</td>
<td>5.82</td>
</tr>
<tr>
<td>Hb F in % of total Hb</td>
<td>79</td>
<td>37</td>
<td>18</td>
</tr>
<tr>
<td>Hb A\textsubscript{2} in % of total Hb</td>
<td>3.5</td>
<td>1.7</td>
<td>2.05</td>
</tr>
<tr>
<td>Blood groups</td>
<td>A</td>
<td>A</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>Rh negative</td>
<td>Rh positive</td>
<td>Rh negative</td>
</tr>
</tbody>
</table>

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**Fig. 3.**—Agar gel electrophoresis pH 6.2 of cord blood hemolysate containing Hb F\textsubscript{Roma} and total hemolysate of normal cord blood. Identical aspect of the two hemoglobins.
Fig. 4.—Chromatography of total cord blood hemolysate containing Hb F_{Roma} on Amberlite IRC-50 pH 6.2. The experiment has continued for 2 hours. The fraction F_{Roma} is markedly faster than Hb F.

Fig. 5.—Ultra-violet spectrum of hemoglobin fractions eluted after starch block electrophoresis. Fetal type of U.V. spectrum of the fraction F_{Roma}.
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**Fig. 6.**—Total cord blood hemolysate containing Hb F<sub>Roma</sub> after alkali resistance test (above) and before (below). Starch gel electrophoresis, discontinuous buffer system, pH 8.2, o-dianisidine stain. After alkali resistance test, Hb F<sub>Roma</sub> has the same aspect and concentration as before exposure to alkali.

H, which is a tetramer of beta chains only (\(\beta^A\)),<sup>12</sup> also resulted in the appearance of a new hemoglobin fraction in the same position as that of Hb A<sub>1</sub> (fig. 8).

Further studies for a more detailed characterization of Hb F<sub>Roma</sub> have been impossible, since the stock of the hemoglobin is exhausted.

**COMMENT**

The electrophoretic mobility of Hb F<sub>Roma</sub> at alkaline pH, and its mobility on agar gel and in chromatography could easily promote the classification of this abnormal hemoglobin as Hb Bart's. The behavior of this hemoglobin in the U.V., its progressive reduction in the 1st month of life, and the absence of a similar fraction in the parents of the baby also agree with the same classification.

However, its electrophoretic mobility at acid pH and, above all, the results of hybridization with other hemoglobins are not compatible with such a classification. As a matter of fact, these results have shown that the molecule of Hb F<sub>Roma</sub> is composed of two different types of polypeptide chains and therefore is different from the molecule of Hb Bart's which is known to be a tetramer of gamma chains (\(\gamma^A\)).<sup>13,14</sup> In fact, if Hb Bart's were involved, hybridization with Hb S should produce Hb F in accordance with the equation:

\[
\alpha^A_2\beta^A_2 + \gamma^F_4 = \alpha^A_2\beta^A_2 + \gamma^F_4 + \alpha^A_2\gamma^F_4 + \beta^A_4
\]

Hybridization with Hb L should produce a formation of a fraction of Hb H and a fraction of Hb slower than Hb L according to the equation:

\[
\alpha^L_2\beta^A_2 + \gamma^F_4 = \alpha^L_2\beta^A_2 + \gamma^F_4 + \alpha^L_2\gamma^F_4 + \beta^A_4
\]
And finally, hybridization with Hb H, which is a tetramer of beta chains, should not produce any new hemoglobin fraction.

On the contrary, it has been ascertained that in the first case, Hb F_Roma does not produce any new hemoglobin fraction, while in the second and third cases it produces a fraction which is electrophoretically identical to Hb A1. This latter fact not only proves that two different types of peptide chains are present in Hb F_Roma, but it also leads to the supposition that one of these two types corresponds to the normal alpha chains of human adult and fetal hemoglobin.

The results obtained by the above hybridization experiments agree with those indicated in the following equations:

1. \[ \alpha^A_{2s} + \alpha^R_{2Roma} = \alpha^A_{2s} + \alpha^R_{2Roma} \]
   \[ \text{Hb D} \quad \text{Hb F}_{Roma} \quad \text{Hb S} \quad \text{Hb F}_{Roma} \]

2. \[ \alpha^L_{2l} \beta^A + \alpha^R_{2Roma} = \alpha^L_{2l} \beta^A + \alpha^R_{2Roma} + \alpha^A_{2s} + \alpha^L_{2l} \]
   \[ \text{Hb L} \quad \text{Hb F}_{Roma} \quad \text{Hb L} \quad \text{Hb F}_{Roma} \quad \text{Hb A} \quad \text{Hb L/F}_{Roma} \]

3. \[ \beta^A + \alpha^R_{2Roma} = \beta^A + \alpha^R_{2Roma} + \alpha^A_{2s} + \gamma^A_{Roma} \]
   \[ \text{Hb H} \quad \text{Hb F}_{Roma} \quad \text{Hb H} \quad \text{Hb F}_{Roma} \quad \text{Hb A} \]

It therefore seems logical to infer that in Hb F_Roma, the alpha chains are normal and that the specific structural alteration resides in the non-alpha chains.
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Fig. 8.—Hybridization experiments of HB $F_{Roma}$ with HB H and with HB $L_{Ferrara}$. Discontinuous buffer system, pH 8.2, amido-Schwartz stain. Both the hybridizations give origin to a new hemoglobin which has a mobility identical to that of HB $A_1$.

The fetal type of the absorption curve in the ultraviolet and the alkali-resistance of HB $F_{Roma}$ shows that the non-alpha chains have the characteristics of the gamma chains, while another fact (the presence of HB $F_{Roma}$ at birth and its gradual disappearance during the first months of life, as HB F became reduced) fully agrees with the hypothesis that HB $F_{Roma}$ is the expression of a mutation at the gamma locus.

With this interpretation it is possible to give a convincing explanation concerning the absence of HB $F_{Roma}$ in both parents, in whom the gamma locus was now inactive. Consequently, the production of gamma chains, normal or pathologic, is practically non-existent.

The rate of disappearance of the abnormal fraction, HB $F_{Roma}$, was not identical to the rate of disappearance of HB F during the first months of life. We do not consider this observation definitive as it is based on only two intermediate determinations before the disappearance of the fraction $F_{Roma}$.

SUMMARY

A new type of abnormal fetal hemoglobin, identified in Rome from the cord blood hemolysate of a healthy newborn girl, is described. This abnormal hemoglobin, which the authors provisionally name HB $F_{Roma}$, has an
electrophoretic mobility at alkaline pH identical to that of Hb Bart’s and a spectrum in the U. V. of the fetal type. It is, however, composed of normal alpha chains and altered gamma chains. Hb F_{Roma}, which was present at birth in the portion of 17 per cent, disappeared completely during the 5th month of life. No abnormal hemoglobins were identified in the parents.

**Summario in Interlingua**

Es describite un nove typo de anormal hemoglobina fetal, identificate in Roma in le hemolysato de sanguine de cordon de un normal neonata. Iste hemoglobina anormal (que le autores ha provisorimente designate como Hb F_{Roma}) possede un mobilitate electrophoretic a pH alcalin identic con illo de Hb Bart’s e un spectro in le region ultraviolette del typo fetal. Tamen, illo es compomite de normal catenas alpha e alterate catenas gamma. Hb F_{Roma}, presente al nascentia in un proportion de 17 pro cento, dispareva completemente durante le quinte mense del vita. Nulle hemoglobinas anormal esseva identificate in le parentes.

**REFERENCES**

A NEW VARIANT: HB F\textsubscript{Roma}

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