Interaction of Hemoglobin Lepore with Sickle Cell Trait and Microcythemia (Thalassemia) in a Southern Italian Family

By Ezio Silvestroni, Ida Bianco and Corrado Baglioni

HEMOGLOBIN LEPORE (Hb Lepore) is a human hemoglobin variant, first described by Gerald and Diamond. Hb Lepore can be separated from human adult hemoglobin (Hb A) by starch block or starch gel electrophoresis. The electrophoretic mobility of Hb Lepore at pH 8.6 is identical to that of Hb S, Hb D and Hb P.

Hb Lepore is characteristically present in heterozygotes for the Hb Lepore gene in relatively small amounts (8–12 per cent); peculiar to this hemoglobin is its constant association with a hematologic picture identical to that of microcythemia (thalassaemia minor).

Following the report by Gerald and Diamond on Hb Lepore in a family of Italian extraction, this hemoglobin has been described in two more families of Italian extraction by Gerald et al. and by Baglioni and in a family of New Guinea aborigines by Neeb et al. An abnormal hemoglobin designated Hb Pylos, very similar if not identical to Hb Lepore, has been reported in several Greek subjects by Fessas et al. Minor differences between Hb Pylos and Hb Lepore, such as the slightly different resolution by paper electrophoresis and column chromatography reported by Fessas et al. are probably to be ascribed to differences in the analytical technics employed, rather than to real differences between these hemoglobins.

The human hemoglobins are made up of two pairs of peptide chains; Hb A is made up of two $ and two $ chains, hemoglobin A2 (Hb A2) is made up of two $ and two $ chains (see Baglioni for refs.). Changes in the hemoglobin molecule can be analyzed by “fingerprinting,” following the technic introduced by Ingram. It has recently been shown that the Hb Lepore from two families of Italian extraction (designated Hb Lepore) gave fingerprints different from those of the Hb Lepore (designated Hb Lepore) obtained from New Guinea aborigines. Both hemoglobins are made up of normal $ peptide chains. The non-$ chains of these hemoglobins show some peptides characteristic of the $ peptide chain and some peptides characteristic of the $ peptide chain; the non-$ chains of Hb Lepore are, however, different from the non-$ chains of Hb Lepore. Baglioni has shown that the non-$ chains of Hb Lepore are made up of a $-like N-terminal amino acid sequence and of a $-like C-terminal amino acid sequence. Baglioni has suggested a similar interpretation for the structure of the non-$

From the Centro di Studi della Microcitemia e delle Anemie Microcitemiche, Istituto d’Igiene, Città Universitaria, Rome, Italy and the International Laboratory of Genetics and Biophysics, Naples, Italy.

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chains of Hb Lepore\text{Hollandia}. The length of the 8-like portion of these chains appears, however, to be shorter than in Hb Lepore\text{Boston}.

Two types of hereditary anemia associated with the presence of Hb Lepore have so far been described: the combination Hb Lepore\text{Boston/microcythemia}\textsuperscript{1,3} and the homozygous Hb Lepore\text{Hollandia} condition.\textsuperscript{6} Similar cases have been reported by Fessas et al.\textsuperscript{7} the homozygous Hb Pylos condition and the Hb Pylos/microcythemia combination. These hereditary anemias are extremely similar to \textit{thalassaemia major} (Cooley's disease) in their clinical manifestations, which are slightly less severe than those of the common Cooley's disease.

We wish to report on the combination sickle cell hemoglobin (Hb S)/Hb Lepore, which has been observed in two members of an Italian family from Campania by Silvestroni and Bianco.\textsuperscript{12} The combination Hb Lepore/microcythemia has again been observed in one member of the same family.\textsuperscript{13} The Hb Lepore from individuals of this family has been studied in detail and characterized by fingerprinting.

**METHODS**

Conventional hematologic methods have been used for blood parameter determinations. The osmotic resistance of erythrocytes has been tested following the method previously described.\textsuperscript{14} Erythrocytes were examined for sickling after a few hours incubation at 37°C. The hemoglobins have been studied by the following techniques:

1. Paper electrophoresis at pH 8.6 and 6.2 using glycine/NaOH and phosphate buffers, respectively.\textsuperscript{15}
2. Starch block electrophoresis in glycine-NaOH buffer at pH 8.6.\textsuperscript{15}
3. Starch gel electrophoresis in the discontinuous buffer system pH 8.6 of Poulik.\textsuperscript{16}
4. Agar gel electrophoresis in citrate buffer pH 6.0.\textsuperscript{17}
5. Column chromatography on ambersite resin IRC-50 according to Huisman and Prins.\textsuperscript{18}
7. Determination of the alkali-resistant hemoglobin fraction by the 1-minute denaturation method of Singer et al.\textsuperscript{19}
8. Solubility of hemoglobin following the method of Itano.\textsuperscript{20}
9. Hybridization of the abnormal hemoglobin with hemoglobins with known alterations in the α or β chain according to Itano and Robinson,\textsuperscript{21} following the microscale adaptation of Gammack et al.\textsuperscript{22}
10. Fingerprinting of the abnormal hemoglobin following the method of Ingram\textsuperscript{9} as modified in the chromatography step by Baglioni.\textsuperscript{23}
11. Fingerprinting of hemoglobin carboxymethylated and digested according to Baglioni.\textsuperscript{8}

**RESULTS**

**Family Studies**

Roman and arabic numerals refer to figure 1 and table 1.

(III-1) The 6-year-old propositus F. R. was referred to one of us (E. S.) because of severe anemia and splenomegaly present since the first few months of life; he has been transfused repeatedly. The child F. R. exhibited the following physical findings: paleness, typical mongoloid facies, very poor general condition, edematous lower limbs, massive hepatosplenomegaly. X-ray examination showed thickening and microareolar osteoporosis of the skull.
diaploe as well as diffuse osteoporosis of the small bones. The hematologic picture of the child F. R. has already been described in detail by Silvestroni and Bianco.12

The diagnosis of *thalassaemia major* (Cooley’s disease) was postulated on the basis of the clinical and hematologic findings. The child was splenectomized after several blood transfusions; the spleen weighed 580 Gm. After splenectomy there was a general improvement and the need for blood transfusions decreased markedly. No abnormal hemoglobin was detected in the blood of F. R. before splenectomy, presumably because the child’s blood was completely substituted for by transfused blood.

A few months after splenectomy F. R. showed a hematologic picture typical of splenectomized *thalassaemia major* patients. The child showed 17 per cent Hb F and a small amount of an abnormal hemoglobin (fig. 2) with the electrophoretic mobility expected for Hb Lepore.

(II-3) The father of the propositus32 showed a hematologic picture typical of microcythemia.

(II-2) The mother of the propositus8 had had severe bone and joint pain accompanied by increase in temperature suffered since childhood. She had nine pregnancies with six miscarriages and only two children were living. The pregnancies were characterized by severe anemia and she required several blood transfusions. Observed a few months after the last pregnancy, she was found to be pale and demonstrated hepatosplenomegaly and cardiac enlargement. The hematologic picture was characteristic of the microdyspanocytic disease as described by Silvestroni and Bianco.29-33 The electrophoretic study of the hemoglobin showed the presence of one main hemoglobin component only, with electrophoretic mobility of Hb S. The solubility of the hemoglobin was very low.

(III-3) The 4½-month-old brother of the propositus showed a hematologic
Table 1.—Hematologic and Hemoglobin Data of Members of F. Family

<table>
<thead>
<tr>
<th>Case</th>
<th>Hb (Gm. %)</th>
<th>RBC (10^6 /mm.3)</th>
<th>WBC (mm.3)</th>
<th>Retic. %</th>
<th>Morph. Alter.</th>
<th>Erythroblasts % WBC</th>
<th>MCV (c.u.)</th>
<th>Beginning hemolysis</th>
<th>Total hemolysis</th>
<th>Bilirubin, mg. %</th>
<th>Total Indir.</th>
<th>Solubility Hb Gm./ml</th>
<th>Hb F %</th>
<th>Hb A2 %</th>
<th>Hb Lepore %</th>
<th>Hb S %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. Raffaele (III-1)</td>
<td>8</td>
<td>3.9</td>
<td>8,600</td>
<td>11</td>
<td>+++</td>
<td>2</td>
<td>---</td>
<td>0.22</td>
<td>0.44</td>
<td>1.32</td>
<td>1.17</td>
<td>2.23</td>
<td>2.31</td>
<td>---</td>
<td>---</td>
<td>---</td>
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<tr>
<td>Before splenectomy</td>
<td></td>
<td></td>
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<tr>
<td>After splenectomy:</td>
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</tr>
<tr>
<td>Two months after</td>
<td>7.2</td>
<td>2.5</td>
<td>18,400</td>
<td>33</td>
<td>+++</td>
<td>130</td>
<td>30</td>
<td>84</td>
<td>0.16</td>
<td>0.38</td>
<td>---</td>
<td>17.12</td>
<td>2.00</td>
<td>present</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>blood transfusion</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Immediately after</td>
<td>8.6</td>
<td>3.5</td>
<td>9,600</td>
<td>8</td>
<td>+++</td>
<td>7</td>
<td>---</td>
<td>4.61</td>
<td>2.29</td>
<td>4.40</td>
<td>---</td>
<td>2.42</td>
<td>4.40</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>blood transfusion</td>
<td>13</td>
<td>5.6</td>
<td>6,800</td>
<td>2</td>
<td>+++</td>
<td>---</td>
<td>---</td>
<td>2.42</td>
<td>0.44</td>
<td>0.65</td>
<td>---</td>
<td>2.42</td>
<td>4.40</td>
<td>---</td>
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</tr>
<tr>
<td>T. Maria (II-2)</td>
<td>9</td>
<td>3.7</td>
<td>10,200</td>
<td>8</td>
<td>+++</td>
<td>4</td>
<td>1</td>
<td>present</td>
<td>80</td>
<td>0.24</td>
<td>1.25</td>
<td>0.72*</td>
<td>9.27</td>
<td>1.78</td>
<td>present</td>
<td>89</td>
</tr>
<tr>
<td>T. Ada (II-4)</td>
<td>8</td>
<td>3.2</td>
<td>7,400</td>
<td>8</td>
<td>+++</td>
<td>---</td>
<td>---</td>
<td>83</td>
<td>0.22</td>
<td>0.42</td>
<td>1.45</td>
<td>0.64*</td>
<td>9.65</td>
<td>0.89</td>
<td>present</td>
<td>99</td>
</tr>
<tr>
<td>F. Maurizio (III-3)</td>
<td>12.8</td>
<td>5.6</td>
<td>7,600</td>
<td>2</td>
<td>+++</td>
<td>---</td>
<td>---</td>
<td>71</td>
<td>0.24</td>
<td>0.42</td>
<td>---</td>
<td>20.28</td>
<td>11.11</td>
<td>16.22</td>
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<tr>
<td>T. Amedeo (II-1)</td>
<td>15.4</td>
<td>6.0</td>
<td>6,600</td>
<td>3</td>
<td>+++</td>
<td>---</td>
<td>---</td>
<td>80</td>
<td>0.26</td>
<td>0.42</td>
<td>---</td>
<td>4.28</td>
<td>1.62</td>
<td>15.82</td>
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<tr>
<td>P. Elvira (I-3)</td>
<td>12.5</td>
<td>6.3</td>
<td>7,000</td>
<td>2</td>
<td>+++</td>
<td>---</td>
<td>---</td>
<td>78</td>
<td>0.26</td>
<td>0.42</td>
<td>---</td>
<td>2.48</td>
<td>2.18</td>
<td>11.20</td>
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<tr>
<td>P. Elisabetta (I-2)</td>
<td>13.0</td>
<td>4.1</td>
<td>7,200</td>
<td>2</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>105</td>
<td>0.38</td>
<td>0.48</td>
<td>---</td>
<td>1.62</td>
<td>1.78</td>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>P. Amedeo (I-8)</td>
<td>12.6</td>
<td>4.2</td>
<td>6,800</td>
<td>2</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>102</td>
<td>0.30</td>
<td>0.48</td>
<td>1.07*</td>
<td>1.10</td>
<td>1.36</td>
<td>---</td>
<td>32.9</td>
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<tr>
<td>T. Elettra (I-7)</td>
<td>14.2</td>
<td>4.3</td>
<td>7,400</td>
<td>1</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>100</td>
<td>0.32</td>
<td>0.46</td>
<td>0.98*</td>
<td>1.78</td>
<td>1.77</td>
<td>34.5</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>T. Marietta (I-6)</td>
<td>16.0</td>
<td>5.0</td>
<td>6,400</td>
<td>2</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>88</td>
<td>0.35</td>
<td>0.50</td>
<td>1.35*</td>
<td>1.56</td>
<td>1.53</td>
<td>---</td>
<td>---</td>
<td>39</td>
</tr>
</tbody>
</table>

*As amorphous ferrohemoglobin at 25 C. in 2.24 M phosphate buffer.
†As amorphous ferrohemoglobin at 25 C. in 2.60 M phosphate buffer.
Fig. 2.—Paper electrophoresis (glycine buffer pH 8.6); bromophenol blue stain. Hemoglobin of a microcythemic individual (above); hemoglobin of the Hb Lepore carrier II-1 (below). Note the clearcut separation of Hb Lepore from Hb A1. nhp = non heme protein.

picture typical of microcythemia. He was a Hb Lepore carrier and has 20 per cent Hb F.

(II-4) The maternal aunt of the propositus showed a hematologic and clinical picture identical to that of her sister (II-2). The electrophoretic analysis of the hemoglobin also showed a pattern identical to that of her sister.

(I-3) The maternal grandmother of the propositus showed a hematologic picture characteristic of microcythemia. She was a Hb Lepore carrier.

(1-6, 1-7 and 1-8) Healthy Hb S carriers.

(I-2) A healthy individual with normal hematologic picture and hemoglobin pattern.

Electrophoretic and Chromatographic Studies

The hemoglobins of the members of the family under investigation were studied as described in Methods. The hemoglobin of the individuals I-3, II-1 and III-3 was separated by starch block and starch gel electrophoresis into a major component, corresponding to Hb A, and into a second component with electrophoretic mobility intermediate between that of Hb A and that of Hb A2. This abnormal hemoglobin showed an electrophoretic mobility identical to that of Hb S by either paper (fig. 2) or starch gel electrophoresis at pH 8.6. One band corresponding to the abnormal hemoglobin was separated from Hb A by chromatography on column of Amberlite IRC-50; this band contained both the abnormal hemoglobin and Hb A2 since its position corresponded to that of Hb A2 from normal individuals. The abnormal hemoglobin has been identified as Hb Lepore on the basis of its electrophoretic and chromatographic behavior as well as on the basis of the fingerprinting pattern (see below).

The hemoglobin of II-2 and II-4 was separated by starch block or starch gel electrophoresis (fig. 3) into a main component with the electrophoretic mobility of Hb S and into a minor component corresponding to Hb A2. On
Fig. 3.—Starch gel electrophoresis in discontinuous buffer (pH 8.6); amido black stain. Hemoglobin of the Hb Lepore carrier II-1 (center); hemoglobin of the 2 sisters (II-2 and II-4) with the Hb S/Hb Lepore disease (above and below). Note the identical electrophoretic mobility of Hb S and Hb Lepore. nhp = non heme protein.

column of Amberlite IRC-50 the hemoglobin of II-2 and II-4 separated into a small fast running band corresponding in mobility to Hb F, into a second band corresponding to the abnormal hemoglobin observed in I-3, II-1 and III-3, and into a main fraction corresponding to Hb S. In agar gel electrophoresis, in addition to the main component in the position of Hb S, two spots were observed; one in the position of Hb F and one with electrophoretic mobility intermediate between that of Hb F and that of Hb S, in the position of Hb A (fig. 4). By running in agar gel electrophoresis the main component isolated by starch block electrophoresis (fig. 5), it was shown that this last hemoglobin fraction corresponds to Hb Lepore and is not Hb A. This main component which runs as a single band in starch block electrophoresis, separated into three components in agar gel electrophoresis. It was thus clearly shown that Hb A was absent in II-2 and II-4 and that Hb Lepore was present.

The amount of Hb Lepore present in carriers varied between 10 and 15 per cent with an average value of 12 per cent. In the propositus III-1, the amount of Hb Lepore present was lower, presumably because of the dilution with transfused hemoglobin. Hb Lepore was barely detectable, only when the patient had not received any blood transfusion for several months. Even then the quantity was not measurable. The level of Hb A₂ in carriers of Hb Lepore, in the individuals with the combination Hb S/Hb Lepore, and in the propositus was within normal limits. The level of Hb F was between 2 and 4 per cent in healthy Hb Lepore carriers; it was about 10 per cent in the 2 individuals with the Hb S/Hb Lepore combination; 2-4 per cent in
Fig. 4.—Agar gel electrophoresis in citrate buffer pH 6.0. Hemoglobin of II-2, affected by the Hb S/Hb Lepore disease (above); hemoglobin of a sickle cell trait carrier (below). Note the presence of a hemoglobin fraction, identified as Hb Lepore, between Hb F and Hb S (above).

Fig. 5.—Agar gel electrophoresis in citrate buffer pH 6.0. Main hemoglobin fraction separated by starch block electrophoresis of the hemoglobin of II-4 (above); total hemoglobin of the same subject (below); for explanations see text.

the propositus after blood transfusion and 17 per cent between transfusions. The brother of the propositus, who was 4½ months old demonstrated a level of 20 per cent Hb F (it was not possible at the time the study was carried out to ascertain whether he has inherited the Hb Lepore gene only or the Hb Lepore/microcythemia combination, like his brother.)

Hybridization Studies

The abnormal hemoglobin, which has been identified as Hb Lepore, has been isolated from the carriers I-3, II-1 and III-3 by starch block electrophoresis. The abnormal peptide chain of this hemoglobin has been identified by the following hybridization experiments.21-22

(1) A new hemoglobin and Hb A (fig. 6) were formed by dissociation and recombination of Hb Lepore and one type of Hb N which is altered in the α peptide chain.24 The formation of the new hemoglobin can be explained in
Fig. 6.—Hybridization of Hb Lepore with Hb N. Starch gel electrophoresis in discontinuous buffer (pH 8.6); amido black stain. After hybridization (center) a new hemoglobin fraction appears between Hb N and Hb Lepore, which does not show in the untreated mixture of these hemoglobins (above) and has electrophoretic mobility identical to that of Hb A\(_1\) (below).

The following way (the non-\(\alpha\) chains of Hb Lepore\(_{\text{Boston}}\) are here designed \(\beta-\delta\):)

\[
\frac{\alpha_2 \beta-\delta_2}{2} + \frac{N \beta_2}{2} = \frac{\alpha_2 \beta-\delta_2}{2} + \frac{N \beta_2}{2} + \frac{\alpha_2 \beta_2}{2} + \frac{N \beta-\delta_2}{2}
\]

Charge difference with Hb A:

\[
\begin{array}{cccc}
+2 & -2 & +2 & -2 \\
0 & 0 & 0 & 0
\end{array}
\]

(2) Similarly Hb A and a new hemoglobin were formed by dissociation and recombination of a mixture of the abnormal hemoglobin and Hb Lerrara which is altered in the \(\alpha\) peptide chain.\(^{25}\)

(3) No new hemoglobin was formed by dissociation and recombination of a mixture of Hb S and Hb Lepore\(_{\text{Boston}}\).

It is thus clearly shown that the abnormal hemoglobin under investigation is made up of normal \(\alpha\) peptide chains and that some structural alteration is present in the non-\(\alpha\) peptide chains.

**Fingerprinting Studies**

The abnormal hemoglobin isolated from the carriers I-3, II-1 and III-3 by starch block electrophoresis was concentrated to approximately 10–15 mg./ml. by ultrafiltration. The hemoglobin solution was dialyzed against distilled water and the globin was prepared according to Rossi-Fanelli et al.\(^{26}\) The globin was digested with trypsin as previously described either directly or after carboxymethylation.\(^{3}\) Fingerprints of the tryptic digests were compared to fingerprints of authentic Hb Lepore\(_{\text{Boston}}\) and to fingerprints of carboxymethylated globin-Lepore\(_{\text{Boston}}\). The fingerprints of the hemoglobin under study were
identical to those obtained from authentic Hb Lepore\textsubscript{Boston}. However, since the trypsin-resistant “core”\textsuperscript{9} is precipitated at the end of the tryptic digestion of hemoglobin, it has been necessary to fingerprint the carboxymethylated globin-Lepore under investigation to show that it is identical to Hb Lepore\textsubscript{Boston}. After carboxymethylation the “core” of the $\beta$ or $\delta$ chain stays in solution and shows up in fingerprints.\textsuperscript{27} Good results were obtained by fingerprinting the carboxymethylated globin-Lepore in the solvent system described by Baglioni.\textsuperscript{23} A photograph of the fingerprints of carboxymethylglobin Lepore and of carboxymethylglobin A is shown in figure 7. The legend of figure 7 illustrates the differences observed between these two fingerprints.

The fingerprinting pattern of corboxymethylglobin A and of carboxymethylglobin Lepore has been described by Baglioni;\textsuperscript{5} the patterns observed in the present investigation are identical to those previously reported. Since the fingerprinting pattern of Hb Lepore\textsubscript{Boston} is rather peculiar, there is little or no doubt that the abnormal hemoglobin under investigation is identical to Hb Lepore\textsubscript{Boston}.

**Discussion**

In the present investigation Hb Lepore has been separated from Hb A by paper electrophoresis at pH 8.6 and by column chromatography on Amberlite IRC-50; Hb Pylos behaves in the same way. This behavior and the identical fingerprints obtained from the Hb Lepore under study and from Hb Lepore\textsubscript{Boston}\textsuperscript{11} allow us to identify these hemoglobins as a single type of hemoglobin, which according to the recommendations on hemoglobin nomenclature should be named Hb Lepore\textsubscript{Boston}.

The most peculiar characteristics associated with the presence of Hb Lepore are the hematologic picture identical to that of microcythemia (hypochromia, microcytosis, increased osmotic resistance and morphologic alterations of the red cells); the presence of a relatively small amount of Hb Lepore (12–15 per cent) in carriers: a normal or decreased level of Hb A\textsubscript{2}; the severe hemolytic anemia resulting from the interaction with microcythemia or from the homozygous Hb Lepore condition\textsuperscript{1,3,7} with a clinical symptomatology similar to that of thalassemia major (Cooley’s disease). The observations reported in this paper confirm all the characteristics indicated above. Hb F is present in III-1 in relatively large amounts, as in other patients with Hb Lepore/microcythemia; the level of Hb F is, however, somewhat lower than that observed in thalassemia major patients.

The observation of 2 individuals (II-2 and II-4) with the Hb S/Hb Lepore\textsubscript{Boston} association is of genetic and pathologic interest. The absence of Hb A in these individuals and genetic studies suggest that the Hb Lepore\textsubscript{Boston} gene is allelic to the $\beta$ peptide chain gene. Stamatoyannopoulos and Fessas\textsuperscript{28} have recently reported that Hb A is absent in 2 individuals with Hb Pylos/Hb S combination. These seem to the authors the first clearcut genetic indications so far reported for the allelism of the $\beta$ peptide chain gene with the Lepore and Pylos genes. This allelism is in agreement with the postulated molecular structure of Hb Lepore\textsubscript{Boston}.
Fig. 7.—Photographs of fingerprints of carboxymethylated globin-Lepore (a) and globin-A (b). The arrows (except 2) indicate peptides, which are different between Hb Lepore and Hb A. 1 indicate peptide LeporeTpII (a) and βTpII (b); 2, LeporeTpXII and βTpXII respectively; 3, LeporeTpIII and βTpIII; 4, LeporeTpX,XI and βTpX,XI; 5, Lepore TpV oxidized and βTpV oxidized, and 6, LeporeTpV and βTpV. For explanations see text. The lysine spot of Lepore (in the lower left corner) is partly off the picture.
HB LEPORE, SICKLE CELL TRAIT AND MICROCYTHEMIA

The Hb Lepore gene either alone or in combination with the Hb S gene manifests itself as a microcythemia gene. Thus, the Hb S/Hb Lepore disease is very similar to the microdrenpanocytic disease of Silvestroni and Bianco29,33 (Hb S/microcythemia association) or only slightly less severe than this disease. The two diseases can not be distinguished from a hematologic point of view. However, small amounts of Hb A may be present in the Hb S/$ microcythemia combination, whereas Hb A is completely absent in the Hb S/Hb Lepore disease; the Hb A2 level is increased in the former and within normal levels in the latter.

The Hb Lepore in patients with Hb S/Hb Lepore disease travels with Hb S in starch gel, paper and starch block electrophoresis. Only in agar gel electrophoresis does Hb Lepore separate from Hb S; it cannot be distinguished from Hb A; however, under these conditions, only by combining paper, starch gel or starch block electrophoresis with agar gel electrophoresis can Hb Lepore be detected. By this technic the Hb Lepore present in the 2 individuals with the Hb S/Hb Lepore disease has been estimated to be approximately 10 per cent of the total hemoglobin. Also in the 2 cases of Hb Pylos/Hb S combination,28 Hb Pylos has been identified as a fraction corresponding to approximately 10 per cent of the total hemoglobin, which migrated on paper with Hb S and on agar with Hb A.

The fingerprinting analysis strongly suggests that the non-α chains of Hb Lepore are manufactured by a hybrid β-δ gene.11 It is likely that this gene has been formed by an unequal nonhomologous crossing over.11 The synthesis of relatively small amounts of Hb Lepore in carriers is probably due to the relative inefficiency of the β-δ gene. This inefficiency of the β-δ gene results in the production of only small amounts of Hb Lepore; as a consequence the total hemoglobin is below normal levels and the red cells are smaller. This pathogenetic mechanism is identical to that postulated by several authors for microcythemia; the inefficiency of either α or β genes causes the α or β-microcythemia. Whether this lack of function of the microcythemic genes is due to changes in the peptide chains similar to those reported for abnormal hemoglobin, or whether a completely different mechanism is responsible, is not yet clear (see discussion by Silvestroni and Bianco).

The family described in this paper provides the first example of Hb Lepore so far described in Italy. The first two families with Hb Lepore were, however, of Italian extraction. It has been reported that in the Greek population, 2 per cent of the people with microcythemic hematologic picture are Hb Pylos carriers.7 The frequency of Hb Lepore carriers may thus be statistically significant in a population where the frequency of microcythemia is high. This observation suggests that a common selective mechanism favors both microcythemic genes and Hb Lepore genes in the same population; it may thus be of interest to measure the frequency of Hb Lepore in some regions of Italy where the frequency of microcythemia is higher.

SUMMARY

An abnormal hemoglobin has been observed in a southern Italian family together with sickle cell hemoglobin and thalassemia (microcythemia). The
A hematologic study has shown that all the carriers of the abnormal hemoglobin are microcythemic. The hemoglobins of these individuals have been studied by paper, starch gel, starch block and agar gel electrophoresis. The abnormal hemoglobin has been isolated and identified by fingerprinting as Hb Lepore

The combination of Hb Lepore
with thalassemia and sickle cell trait has been observed in members of this family. The hematologic and biochemical implications of the Hb Lepore/thalassemia disease are discussed.

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Prof. E. Silvestroni, M.D., Director, Division of Hematology, St. Eugene Hospital, Rome; Director of Research Center for Microcythemia, Department of Hygiene, University of Rome, Rome, Italy.

Prof. I. Bianco, M.D., Assistant, Research Center for Microcythemia, Department of Hygiene, University of Rome, Rome, Italy.

C. Baglioni, M.D., International Laboratory of Genetics and Biophysics, Naples, Italy.
Interaction of Hemoglobin Lepore with Sickle Cell Trait and Microcythemia (Thalassemia) in a Southern Italian Family

EZIO SILVESTRONI, IDA BIANCO and CORRADO BAGLIONI