Hb Potomac (101 Glu → Asp): Speculations on Placental Oxygen Transport in Carriers of High-Affinity Hemoglobins

By S. Charache, R. Jacobson, B. Brimhall, E. A. Murphy, P. Hathaway, R. Winslow, R. Jones, C. Rath, and Joan Simkovich

Blood from a woman with unexplained erythrocytosis had increased oxygen affinity, but no abnormality could be detected by electrophoresis or chromatography of her hemolysate. Separation of the tryptic peptides of her β chains disclosed two half-sized peaks in the region of β T-11. The faster of these was abnormal, with the structure β 101 Glu → Asp. The new hemoglobin was called “Potomac.” Three of the proband’s four surviving siblings and both of her children were carriers. Differences in the ratio of carrier:normal children born to male or female carriers of 23 other high-affinity hemoglobins were not significant. The high proportion of carriers in this kindred was probably due to chance alone, and not because high maternal oxygen affinity interfered with oxygen transport to fetuses with normal hemoglobin.

Inherited or acquired abnormalities of oxygen affinity do not play a critical role in oxygen transport in healthy, resting subjects at sea level, for a variety of compensatory mechanisms are used to restore homeostasis. When reserves are exhausted or stressed, oxygen affinity becomes much more important. Such situations include heart failure, severe anemia, high altitude, severe exercise, and pregnancy.

Textbooks of obstetrics point out that transport of oxygen from mother to fetus is aided by the difference in their oxygen affinities. Most mammals show such a difference, often greater than that in man—although the domestic cat and (in early pregnancy) the elephant do not. In 1967 Moore et al. pointed out that the usual difference in affinity between adult and fetal hemoglobin is not necessary for delivery of a healthy human fetus. A female carrier of a high-affinity hemoglobin (Hb Zurich, P50 21.6 mm Hg) had a hematologically normal child, although the difference in P50* across the placenta had probably only been 1.1 mm Hg rather than the usual 6+ mm Hg (normal maternal P50 26.6, fetal 20.5 mm Hg). Parer made the same point in 1970, this time in a carrier of Hb Rainier (P50 12.5 mm Hg).

*P50: the oxygen pressure in mm Hg necessary for 50% saturation of hemoglobin, corrected to pH 7.4 and 37°C unless specified to the contrary.

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Since then a number of hemoglobins with much higher affinity than Hb Zurich have been described: in some [for instance, a family with hemoglobin Osler (P50 11.0 mm Hg)\(^{16}\)] more than the expected 50% of the children of female probands were also carriers, suggesting that when maternal oxygen affinity is very high, reversal of the usual fetomaternal difference in O\(_2\) affinity diminishes the chance for successful completion of pregnancy. Against that hypothesis were the observations that (1) most hemoglobins with very high oxygen affinity have been \(\beta\)-chain variants, and the affinity of carrier fetuses would be expected to be abnormally high only late in pregnancy when \(\beta\)-chain synthesis increases, and (2) spontaneous abortions have been infrequently reported among carriers of high-affinity hemoglobins.

We have recently identified a new high-affinity hemoglobin, which we have called "Potomac" because the proband lives near that river. Both of her children (and three of her four siblings) are also carriers, a segregation ratio which suggests that the high affinity of her blood may have had a selective influence on fetal survival. We report here data on the structure of the new hemoglobin. We have been unable to separate it from Hb A, and data on the purified component are lacking. We also present a review of pedigrees of carriers of other high-affinity hemoglobins to explore the question as to whether the unusually high proportion of carriers of Hb Potomac is due to chance alone.

**MATERIALS AND METHODS**

**Methods**

Hematologic, electrophoretic, and chromatographic procedures, and measurement of O\(_2\) affinity of whole blood were carried out as described previously.\(^{17}\) Additionally, continuous whole blood oxygen equilibrium curves were measured at constant pH and P\(_{\text{CO}_2}\) by the slow addition of H\(_2\)O\(_2\) to deoxygenated blood samples.\(^{18}\) Blood samples were anticoagulated with EDTA, and measurements of whole blood O\(_2\) affinity were performed within 2 hr of blood collection. The oxygen affinity of hemolysates was measured by the method of Imai et al.\(^{19}\) using 0.05 M bis-Tris-0.1 M NaCl buffers.

The affinity of samples of whole blood, collected in EDTA-containing Vacutainers and mailed to Baltimore, Md., from other cities without refrigeration, was estimated from measurements on unstripped dilute hemolysates (1.5 x 10\(^{-5}\) M Hb) to which 2,3-diphosphoglycerate (DPG) was added to give a final concentration of 1.4 x 10\(^{-4}\) M (if no DPG was present in the original sample). Inositol hexaphosphate (IHP) was added to other samples to yield the same final concentration. A methemoglobin-reducing system\(^{20}\) was added in every case. When measurements were made in this fashion, the oxygen affinities of hemolysates of erythrocytes from carriers who lived in the Baltimore-Washington area (III-2, for instance) did not differ from those of carriers living in New England (III-3), although the former samples were studied within a few hours of collection and the latter were mailed. Heat stability and denaturation by 17% isopropanol were measured by previously described methods.\(^{21,22}\)

Globin chains were separated by the method of Clegg et al. and then aminoethylated,\(^{23}\) dialyzed, lyophilized, and sent to Portland, Ore. There, the \(\beta\) chains were digested with trypsin and the peptides were separated by the method of Jones.\(^{24}\) Further details of peptide analyses are given in Results in the discussion of structural studies.

**Case Report**

A 27-yr-old woman was found to have a hematocrit of 55% in 1949 at the time of a spontaneous abortion. Three years later her hemoglobin concentration was 18.6 g/dl. Her physician recognized that this value was abnormally high, but she was neither treated nor evaluated for the cause of her erythrocytosis. In 1966, she sought medical advice because of vertigo. The hematocrit was
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Fig. 1. Pedigree of the proband.

57.5%, platelet and white cell counts were normal, and red cell mass was 34 ml/kg. One 500-ml phlebotomy was performed.

She did not return until 2 yr later, when she developed increasing fatigue. Her hematocrit was 58.5% and her red cell mass was 39 ml/kg. White cell and platelet counts and an intravenous pyelogram were normal. She was treated with phlebotomies every 2-4 wk. In 1969, when she was 47 yr old, Dr. J. Simkovich demonstrated that the oxygen affinity of her blood was increased.

She returned in 1976, at the age of 55, because of recurrent vertigo; the hematocrit was 57.8%. The leukocyte alkaline phosphatase score was normal, arterial P\(_{O_2}\) was 85 mm Hg, and red cell mass was 36.4 ml/kg. Removal of 500 ml of blood did not ameliorate her symptoms.

Several members of her family knew that they had erythrocytosis. The pedigree is shown in Fig. 1 and hematologic data are given in Table 1. Patients whose hemoglobin concentrations equaled or exceeded 17 g/dl had low P\(_{50}\) when dilute hemolysates were studied. None of the affected family members had received treatment for a high hematocrit and none had an elevated reticulocyte count.

RESULTS

Oxygen Affinity of Blood

Continuous oxygen dissociation curves of whole blood were biphasic (Fig. 2), suggesting that the sample contained more than one type of hemoglobin; the estimated P\(_{50}\) was 12.5 mm Hg at 37°C, P\(_{CO_2}\) 40 mm Hg, and pH 7.4 (normal 27-29 mm Hg). Independent measurements, using analyzed gas mixtures and a

<p>| Table 1. Hematologic Data From the Proband’s Family |
|----------------|----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Hb (g/dl)</th>
<th>Retic. (%)</th>
<th>P(_{50})* (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-1</td>
<td>56</td>
<td>F</td>
<td>19</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>19</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>F</td>
<td>19</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>F</td>
<td>13.7</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>F</td>
<td>20.9</td>
<td>—</td>
</tr>
<tr>
<td>III-1</td>
<td>21</td>
<td>M</td>
<td>19</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>17</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>F</td>
<td>17.9</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>F</td>
<td>14.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>M</td>
<td>17.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>M</td>
<td>16.4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>M</td>
<td>14.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>M</td>
<td>14.5</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>F</td>
<td>14.9</td>
<td>—</td>
</tr>
<tr>
<td>IV-1</td>
<td>9</td>
<td>M</td>
<td>17.1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>14.2</td>
<td>—</td>
</tr>
</tbody>
</table>

* Hemolysate 0.1 g/dl at 25°C, pH 7.4, 0.05 bis-tris–0.1 M NaCl, in the presence of 1.4 x 10\(^{-4}\) M DPG.
Co-Oximeter, gave a $P_{50}$ of 11.7 mm Hg under the same conditions, with a normal value of 26.3.

Dilute hemolysates from all members of the proband’s family with erythrocytosis also yielded biphasic dissociation curves. The $P_{50}$ of red cell hemolysate of II-5 was remarkably low on duplicate determinations; her hemoglobin concentration was the highest in the kindred. The Bohr effect of hemolysates and reactivity with DPG and IHP, measured at pH 6.5-7.5, were approximately normal (Fig. 3). No difference in Bohr effect was found when the $P_{75}$ of carrier hemolysates (which reflected predominantly the properties of Hb Potomac) were compared with $P_{25}$ (which reflected the properties of HbA).

**Stability**

Fresh hemolysates containing Hb Potomac did not show more precipitation than normal samples when incubated in 17% isopropanol at 37°C or phosphate buffer at 60°C. Hemolysates prepared from mailed blood samples of both carriers and normal family members yielded positive isopropanol tests, although they did not contain methemoglobin.

**Structural Studies**

An abnormal component could not be separated by electrophoresis at pH 8.6 on cellulose acetate or at pH 6.0 on agar. No abnormalities were found after chromatography of hemoglobin on DEAE-Sephadex (Pharmacia Fine Chemicals, Piscataway, N.J.) or Biorex-70 (BioRad Laboratories, Richmond, Calif.)
or chromatography of globin on CM-52 (Whatman, Clifton, N.J.). Amino-
ethylated β chains from the A-Potomac hemolysate were digested with trypsin.
The peptides from 40 mg of tryptic hydrolysate were separated on a 0.9 ×
30 cm column of Aminex A-5 (BioRad) cation-exchange resin. A linear gra-
dient of two buffers, 375 ml each, was used at a flow rate of 30 ml/hr. The first
buffer was 0.05 M pyridine-acetate, pH 3.1; the second was 0.39 M pyridine-
acetate, pH 4.5. The remaining peaks were eluted with 0.5 M pH 5.0 pyridine-
acetate.

This procedure gave a peptide pattern which looked like that of a normal β
chain from HbA, except that two half-sized peaks were eluted near the position
usually occupied by peptide β T-11 (Fig. 4). On amino acid analysis, the faster
of the two peaks proved to have the composition of normal β T-11, except that
it had one less glutamyl residue and one extra aspartyl residue, suggesting that
the replacement had occurred at residue 101 (Table 2). The slower of the two
peaks had the same amino acid analysis as a normal β T-11 peptide.

The aberrant β T-11 peptide was then treated overnight with 0.5 M acetic
acid in an evacuated ampoule at 110°C in order to cleave it at its aspartyl
residues. The resulting solution was chromatographed on a 0.9 × 60 cm col-
umn of Aminex 50W-X2 resin with pyridine-acetate buffers. On amino acid
analysis, the products of cleavage in order of their elution from the column
were found to be: free aspartic acid; free proline; a tripeptide containing Leu,
His, Val; and another containing Asx, Phe, Arg. The sequence of normal β
T-11 and the probable sequence of the abnormal β T-11 peptide from Hb

Table 2. Amino Acid Composition of Normal and Abnormal β T-11 Peptides From
Hb Potomac (Moles/Mole Peptide)

<table>
<thead>
<tr>
<th></th>
<th>Abnormal β T-11</th>
<th>Normal β T-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine (His)</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Arginine (Arg)</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Aspartic acid and asparagine (Asx)</td>
<td>3.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Glutamic acid (Glu)</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Proline (Pro)</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Valine (Val)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Leucine (Leu)</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Phenylalanine (Phe)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Potomac are compared below. (Lines represent cleavage points with 0.5 M acetic acid; see Table 2 for definitions of all abbreviations.)

Residue in Chain

| Normal β T-11 | Leu-His-Val-Asp-Pro-Glu-Asn-Phe-Arg |
| βT-11 from Hb Potomac | Leu-His-Val-Asp-Pro-Asp-Asn-Phe-Arg |

Amino acid analyses were run on the other peptides from the original tryptic hydrolysate and all had normal compositions. Residue 101 was inferred to be aspartic acid (Asp) and not asparagine (Asn) for three reasons: it was cleaved by 0.5 M acetic acid; no charge difference was observed between HbA and Hb Potomac on electrophoresis on cellulose acetate; and the codon for Glu could change to that for Asp with only one nucleotide change, while two would be necessary for the change of Glu to Asn.

Review of Other High-Affinity Hemoglobins

Pedigrees of families with high-affinity hemoglobins were compiled, using published data and details provided by generous personal communications. Twenty-three families were studied; copies of their pedigrees and a detailed analysis were published elsewhere.26

The hypothesis to be tested was that high maternal oxygen affinity decreases the probability that children with normal hemoglobins will be born. The ratio of affected to normal children was compared between carrier females and carrier males (whose wives had normal oxygen affinity). After correction for bias of ascertainment, the probabilities that differences in ratios were due to chance alone were 0.95 for all families and 0.96 for families with P 16 mm Hg, based on analysis of 111 children born to male carriers and 96 born to females.

DISCUSSION

The site of the amino acid substitution (residue G3β in helical notation) in Hb Potomac is at an interface between the α and β chains of hemoglobin.27 Dr. Max Perutz suggests that the high oxygen affinity of Hb Potomac is produced by destabilization of its deoxy structure as follows: The side chain of the normal glutamic acid residue is only 3.5 Å from aspartic acid G1 of the neighboring α chain in the deoxy conformation. If aspartic acid is substituted at G3β, its carboxylated oxygens come within 2.5 Å, close enough to produce strong repulsive forces, distort the deoxy structure, and render it unstable. If the side chain slips into an alternative conformation, which avoids contact with aspartic Gluα, it contacts aspartic acid G1β, with similar results. Equilibrium between the oxy and deoxy conformations is shifted toward the more stable oxy structure, which has intrinsically high affinity, and patients develop erythrocytosis as a result. Electrophoretic mobility of the abnormal hemoglobin is not altered, for glutamic and aspartic acids have like charges at pH 8.6.

Three other substitutions are known at position G3β (Table 3). They produce combinations of increased oxygen affinity, decreased stability, ability to form stable hybrids (αβ^Xβ^X), and electrophoretic mobility different from that ex-
Table 3. Properties of Other Hemoglobins With Substitutions at $\beta$ 101 (G3)

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>Charge Change</th>
<th>Mobility* pH 8.6</th>
<th>Whole Blood $P_{50}$ (mm Hg)</th>
<th>Stability</th>
<th>Stable Hybrid</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alberta</td>
<td>Gly +2</td>
<td>+1.5</td>
<td>15.5</td>
<td>Normal</td>
<td>+</td>
<td>28</td>
</tr>
<tr>
<td>British Columbia</td>
<td>Lys +4</td>
<td>+1</td>
<td>23.2</td>
<td>Normal</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Rush</td>
<td>Glu +2</td>
<td>+1.5</td>
<td>Normal</td>
<td>Low</td>
<td>+</td>
<td>30</td>
</tr>
</tbody>
</table>

*Cellulose acetate electrophoresis: HbS, +1; HbC, +2.

...expected solely on the basis of the change in charge of the amino acid side chain. In Hb Alberta and Hb British Columbia, destabilization of the deoxy conformation may result from introduction of water or excess positive charge into the interior of the molecule. The latter hypothesis has also been advanced to explain the instability of Hb Rush.°

It has recently been suggested that Hb$\alpha_1$, found in increased concentration in diabetics, raises maternal oxygen affinity and hence might pose a threat to the fetus. Our data suggest that if diabetes does increase oxygen affinity of the mother, the hazard to the fetus is not significant.

Review of pedigrees of other families with high-affinity hemoglobins has provided no evidence that being a carrier has survival value for the fetus of an affected mother. Even if placental blood flow, $P_{O_2}$, $P_{CO_2}$, and hemoglobin concentration in the fetus remain normal, the high hemoglobin concentration of the carrier mother would tend to maintain oxygenation. Since there are very few high-affinity $\alpha$-chain mutants, we can conclude only that reversal of the usual gradient in oxygen affinity is not lethal in late pregnancy, when $\beta$-chain synthesis begins. No statement can be made as to whether reversal produces less obvious damage, such as low birth weight or retarded development, for data are insufficient.

ACKNOWLEDGMENT

We thank Dr. Max Perutz for his interpretation of the molecular basis for increased oxygen affinity in Hb Potomac, and Dr. André Hellegers and Dr. Joan Simkovich for their advice.

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Hb Potomac (101 Glu replaced by Asp): speculations on placental oxygen transport in carriers of high-affinity hemoglobins

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