Hypoprothrombinemia: Case Report

By R. R. Montgomery, A. Otsuka, and W. E. Hathaway

A patient with a significant history of spontaneous and posttraumatic bleeding was found to have hypoprothrombinemia. His prothrombin (factor II) activity by clotting assay was 9.5% and his factor II antigen was 5%. Crossed immunoelectrophoresis and sucrose density gradient ultracentrifugation of the patient’s plasma showed his prothrombin to be qualitatively indistinguishable from normal prothrombin by these techniques.

Hereditary deficiency of prothrombin (factor II) is a rare disorder. In the earlier literature the term “true” has been used to denote isolated factor II deficiency in order to distinguish it from a combined deficiency of factor II and other coagulation factors. Since the institution of immunologic methods to study coagulation proteins, investigators have separated them into functional and nonfunctional proteins. With the study of prothrombin deficiencies, patients have been categorized as having hypoprothrombinemia and dysprothrombinemia. Hypoprothrombinemia denotes a deficiency in immunoreactive factor II protein as well as procoagulant activity. Dysprothrombinemia describes a decrease in procoagulant activity with normal or near normal levels of factor II antigen. This report describes a 31-yr-old male with hypoprothrombinemia.

Materials and Methods

Laboratory Studies

Coagulation studies were performed on plasma obtained from citrated whole blood. Nine volumes of blood were drawn into 1 volume of anticoagulant (3 parts 0.1 M sodium citrate and 2 parts 0.1 M citric acid) and centrifuged to obtain platelet-free plasma. Studies included platelet count by direct phase method and bleeding time by a modified Ivy method using a template (incision dimensions 1 mm deep, 5 mm long). Kaolin-activated partial thromboplastin time (PTT), prothrombin time (PT), thrombin time, and assays of factors I, V, VII, VII-X, IX, and XI were performed as previously detailed. Factor VIII was assayed by one-stage, two-stage, and immunologic assays.

Factor II was assayed using the method of Owren and Aas, which was modified to use prothrombin-free bovine plasma. This assay used aged prothrombin-free serum as a source of activated factor X and prothrombin-proconvertin-free bovine plasma as sources of factor V and fibrinogen. Equal amounts of test plasma, prothrombin-free serum, prothrombin-proconvertin-free bovine plasma, and rabbit brain thromboplastin were recalcified and a double logarithmic normal curve was constructed.

Immun assay of factor II protein was performed using a commercial rabbit anti-human factor II antibody (Behring Diagnostics, Somerville, N.J.) and the Laurell method as modified by Zimmerman et al. The antibody concentration was 0.3%, but optimal antibody concentrations had to be determined for each lot of antibody. Holes were cut using a 4.5-mm punch. Serial dilutions of
normal plasma and test plasma were added to the wells in 20-μl aliquots. The peak heights were plotted on semilogarithmic paper and the level of factor II antigen was determined.

Crossed immunoelectrophoresis was performed on 5.1 × 7.6 cm glass slides. Two ml of 0.9% agarose (dissolved in 0.03 M barbital buffer, pH 9.5) was layered on the long edge of the slide by pressing a 3-mm-thick strip of plastic against the slide 1.5 cm from the edge. A well was cut at one end and a 25-μl sample applied. A 5-μl specimen of 1% Evan’s blue dye in 20% bovine serum albumin was added to serve as a marker. The first dimension was subjected to 3.25 mA/slide until the albumin marker migrated 6.5 cm. The second dimension was then prepared using 5 ml of 0.9% agarose in 0.03 M barbital buffer (pH 8.6) and an antibody concentration of 0.3%. Electrophoresis was carried out in the second dimension at 5 mA/slide for 18 hr. The patient’s plasma was compared to a 5% normal plasma pool because of the variation in peak heights due to the patient’s hypoprothrombinemia.

Sucrose density gradient (10%-40%) ultracentrifugation was performed to compare the sedimentation velocity of the prothrombin from the patient, O.M., to a 5% sample of normal plasma. The gradients were eluted from the bottom in 0.3-ml fractions. These fractions were then analyzed by quantitative immunoelectrophoresis for factor II antigen. Fibrinogen, α1-antitrypsin, IgG, and IgM were assayed by radial immunodiffusion and served as internal markers.

Case Report
O.M. was a 31-yr-old Chicano male referred for evaluation of a life-long bleeding disorder. By history, he had excessive bleeding at circumcision and easy bruising throughout childhood. At age 10 he had a large spontaneous hematoma on his thigh. He was hospitalized and required transfusions for excessive bleeding from a dental extraction at age 11. A knife injury to his neck at age 17 developed into a hematoma requiring a tracheostomy. Bleeding was controlled with multiple transfusions of fresh whole blood. He had two dental extractions that bled excessively and did not respond to multiple injections of vitamin K. At age 21 he had a spontaneous hematoma of his left anterior thigh. At that time, liver function tests were normal. At age 22, he sustained a stab wound to the left chest and was hospitalized for several months and required multiple transfusions. He did well until age 26, when he had a spontaneous upper gastrointestinal bleed that ceased after transfusion of 6 units of fresh whole blood. He was first seen and hospitalized at the University of Colorado in September 1971 for an infected hematoma of his foot. At this time, liver function tests were normal. His PT was 20 sec with a control of 13 sec. Further evaluation of his coagulation status was inconclusive at that time.

The patient was first seen in our laboratory in February 1975 for evaluation of his coagulation defect. There was no history of a bleeding diathesis in the family, but family members were not available for study.

RESULTS
Coagulation studies on O.M. are presented in Table 1. The significant studies were the prolonged PTT and PT. Assays of factors VII, VII-X, IX, XI, VIII, and V were all normal. Specific prothrombin activity was 9.5%. Figure 1 shows the factor II antigen determinations from two separate specimens drawn at different times. The amount of prothrombin antigen was 5% of normal on both occasions. A sample of his plasma was mixed 1:1 with normal plasma and his PT became normal. The PT of the mixture did not prolong with incubation at 37°C and thereby showed the lack of a significant inhibitor.

Crossed immunoelectrophoresis was undertaken to compare his prothrombin with normal prothrombin. Figure 2 shows the mobility of the patient’s prothrombin to be indistinguishable from a 5% sample of a pool of normal plasma diluted in barbital saline. In addition, a 5% sample of normal plasma was resuspended in A1(OH)3 absorbed plasma to see if the amount of protein present interfered with the migration on crossed immunoelectrophoresis. All three
Table 1. Coagulation Studies on Patient O.M.

<table>
<thead>
<tr>
<th>Study</th>
<th>Normal Range</th>
<th>O.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count/cu mm</td>
<td>200,000–450,000</td>
<td>195,000</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
<td>2–6</td>
<td>3</td>
</tr>
<tr>
<td>PTT (sec)</td>
<td>37–50</td>
<td>54.8</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>12.5–14</td>
<td>18.2</td>
</tr>
<tr>
<td>TT (sec)</td>
<td>8–10</td>
<td>8.6</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>200–400</td>
<td>280</td>
</tr>
<tr>
<td>VIII, 1 stage (%)</td>
<td>60–150</td>
<td>121</td>
</tr>
<tr>
<td>VIII, 2 stage (%)</td>
<td>60–150</td>
<td>100</td>
</tr>
<tr>
<td>IX (%)</td>
<td>50–140</td>
<td>132</td>
</tr>
<tr>
<td>XI (%)</td>
<td>60–130</td>
<td>120</td>
</tr>
<tr>
<td>VII (%)</td>
<td>60–130</td>
<td>100</td>
</tr>
<tr>
<td>VII-X Complex (%)</td>
<td>60–130</td>
<td>92</td>
</tr>
<tr>
<td>V (%)</td>
<td>65–155</td>
<td>108</td>
</tr>
<tr>
<td>II Activity (%)</td>
<td>60–120</td>
<td>9.5</td>
</tr>
<tr>
<td>II Immunoassay (%)</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>2nd-Stage inhibition screen</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

samples migrated identically, suggesting that the presence of other proteins did not affect the migration of prothrombin by this method. This effect was different from that of other proteins on sucrose density gradients.

Sucrose density gradient ultracentrifugation was done to compare the sedimentation velocity of the patient’s prothrombin to normal prothrombin in intact plasma. Since the normal plasma was diluted to 5%, the effect of diluting fluid was determined. Figure 3 shows the comparison of normal plasma diluted in barbital buffer (pH 7.4) and diluted in Al(OH)₃ absorbed, prothrombin-free plasma. 

![Quantitative immunoelectrophoresis of factor II antigen comparing the patient’s plasma (O.M.) run undiluted (N) to serial dilutions of a normal plasma pool.](image)
normal plasma. The presence of other proteins affected the sedimentation velocity. Therefore, the patient's prothrombin was compared to the 5% normal plasma diluted in Al(OH)₃ absorbed plasma. The results are shown in Figure 4. The sedimentation velocity of the patient's prothrombin and normal prothrombin were identical and corresponded to an S rate of 4.9S.

**DISCUSSION**

With the use of immunologic techniques to evaluate clotting disorders, most deficiencies have been categorized as either deficient production of a protein or production of a nonfunctional molecule. Several instances of hypoprothrombinemia were reported prior to the development of the current immuno-
normal or normal range.

AR, autosomal recessive.

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Fig. 4. Sucrose density gradient ultracentrifugation comparing O.M. (---) to 5% normal plasma resuspended in Al(OH)₃ absorbed plasma (----). Standard curve is shown for sedimentation velocity (S) with α₁-antitrypsin, IgG, and fibrinogen standards (----).

logic assays,¹⁰⁻¹² and thus were not differentiated into hypoprothrombinemia or dysprothrombinemia. There have been no reports of complete absence of prothrombin—either activity or antigen. Table 2 summarizes the cases of “prothrombin deficiency” in which a measure of prothrombin antigen was performed and was found to be normal.¹³⁻¹⁶ The patient described in this report is also included. In addition to these seven patients, a patient first reported by Quick¹⁷ and subsequently studied by Lanchantin and his associates¹⁸ was found to have low levels of prothrombin by chemical separation techniques. All of the patients had spontaneous bleeding problems and those who were challenged by surgery had major postoperative hemorrhagic complications.

In these patients, the inheritance of the abnormality appeared to be autosomal recessive. Their ethnic backgrounds were as follows: four Italian, and one each of Mexican-American, French, and Puerto Rican ancestry. As noted in Table 2, this group of patients had the expected prolongation of both PT and PTT. The patient reported by Kattlove and his associates¹³ had a PT of 14.5 sec

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Ethnic Background</th>
<th>Bleeding History</th>
<th>PT (sec)</th>
<th>PTT (sec)</th>
<th>Factor II (%)*</th>
<th>Bod. Immunol.</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kattlove et al.¹³</td>
<td>30</td>
<td>F</td>
<td>Puerto Rican</td>
<td>Menorrhagia</td>
<td>14.5</td>
<td>50</td>
<td>25.0</td>
<td>25.0</td>
<td>AR</td>
</tr>
<tr>
<td>Josso et al.¹⁴</td>
<td>1970</td>
<td>23</td>
<td>M</td>
<td>French</td>
<td>Epistaxis</td>
<td>13.0</td>
<td>12.5</td>
<td>AR</td>
<td></td>
</tr>
<tr>
<td>Baudo et al.¹⁵</td>
<td>1972</td>
<td>27</td>
<td>M</td>
<td>Italian</td>
<td>Postoperative</td>
<td>17</td>
<td>75</td>
<td>6.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Girolami¹⁶</td>
<td>1971</td>
<td>F</td>
<td>Italian</td>
<td>Moderately severe</td>
<td>15.0</td>
<td>12.5</td>
<td>AR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groll et al.¹⁷</td>
<td>1971</td>
<td>M</td>
<td>Italian</td>
<td>Moderately severe</td>
<td>9.0</td>
<td>11.5</td>
<td>AR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>This report</td>
<td>31</td>
<td>M</td>
<td>Chicano</td>
<td>Spontaneous muscle bleeding</td>
<td>18.2</td>
<td>54.8</td>
<td>9.5</td>
<td>5.0</td>
<td>?</td>
</tr>
</tbody>
</table>

*Percent of normal.
†Normal or normal range.
‡AR: autosomal recessive.
and was found to have a prothrombin level of 25%. The presence of excessive bleeding with only minimal prolongation of the PT is important to note. Most patients had a parallel decrease in immunologic and procoagulant activity.

Crossed immunoelectrophoresis of the patient's plasma (Fig. 2) showed the migration pattern to be identical to that of normal plasma. Since the protein concentration may affect electrophoretic migration in the standard Laurell technique, it was of interest to note the identical mobility of the normal prothrombin when diluted with either Al(OH)₃ absorbed plasma (prothrombin free) or barbital buffer (Fig. 2).

Although the true sedimentation velocity of a protein can only be obtained in the purified state, an evaluation of a protein's behavior in intact plasma may give information as to its sedimentation velocity as well as possible interactions with other plasma proteins. Thus, when normal plasma was diluted in buffer, its distribution was different than when it was diluted in Al(OH)₃ absorbed plasma (Fig. 3). When the patient's plasma was compared to 5% normal plasma diluted in Al(OH)₃ absorbed plasma, an identical distribution and sedimentation rate were obtained. The average sedimentation velocity of prothrombin in normal human plasma and in the patient's plasma was 4.9S. This value agreed with the reported S rate for bovine prothrombin of 4.6S–5.3S.

Table 3 lists the five dysprothrombinemias that have been described. All of these patients had decreased biologic activity of prothrombin, but normal or near normal levels of immunoreactive protein. The data in both Table 2 and Table 3 frequently indicate minimal prolongation of the PT in the face of significant clinical bleeding. Clotting tests are usually most sensitive to the factors involved in the initiation of the clotting cascade and least sensitive to the factors at the conclusion of the clotting process. As Biggs has emphasized, the PT is relatively insensitive to prothrombin deficiency. Since the PT may be normal or only minimally prolonged in hypoprothrombinemia and dysprothrombinemia, evaluations other than simple screening tests in patients who have a strong clinical history of bleeding diathesis are indicated.

Subsequent to our evaluation of this patient he underwent dental surgery and received a single prophylactic transfusion with prothrombin complex concen-

### Table 3. Reports of Dysprothrombinemia

<table>
<thead>
<tr>
<th></th>
<th>Cordeza ²⁴</th>
<th>Barcelona ²⁵</th>
<th>San Juan ²⁶</th>
<th>Padua ²⁷</th>
<th>Brussels ²⁸</th>
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<tbody>
<tr>
<td>PT (sec)</td>
<td>12.2–14.7</td>
<td>—</td>
<td>—</td>
<td>15.5</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>(11.5–13.5)</td>
<td></td>
<td>(13–14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTT (sec)</td>
<td>55–86</td>
<td></td>
<td></td>
<td>45</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>(45–90)</td>
<td></td>
<td></td>
<td>(34–45)</td>
<td></td>
</tr>
<tr>
<td>Prothrombin assays</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic (%)†</td>
<td>†</td>
<td>4–5</td>
<td>15–20</td>
<td>52</td>
<td>35</td>
</tr>
<tr>
<td>Immunologic (%)†</td>
<td>87–125</td>
<td>75–100</td>
<td>normal</td>
<td>100</td>
<td>71</td>
</tr>
</tbody>
</table>

*Normal range.
†Percent of normal.
‡Prothrombin Cardeza reported in units rather than percent of normal. Patients' range was 114–174 U/ml; normal range was 260–330 U/ml.
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rates and had no problems with postoperative bleeding. Factor II survival studies were not performed.

Therapy of patients with hereditary hypoprothrombinemia can be undertaken easily because of the long half-life of prothrombin. In two studies of prothrombin survival, the initial $\tau_{1/2}$ was 0.36 days in one and approximately 1 day in the other, probably due to a distribution phenomenon. After the first 24-hr period, the second-phase $\tau_{1/2}$ was found to be between 2.5 and 3.0 days. The level of prothrombin necessary for normal hemostasis has been estimated at 40%. Prothrombin is very stable in vitro and contains greater than 95% of the prothrombin activity after storage for 42 days. Because of the risk of hepatitis, prothrombin complex concentrates should be used only when circulatory overload would contraindicate the use of single-donor plasma.

REFERENCES

21. Tishkoff GH, Williams LC, Brown DM:


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