Assay of Plasma Thromboplastin Antecedent (PTA) with Artificially Depleted Normal Plasma

By Herbert I. Horowitz, Winthrop P. Wilcox and Mitsu M. Fujimoto

ROSENTHAL ET AL. in 1953 described a familial hemorrhagic disorder distinct from classical hemophilia and involving women as well as men. The disorder was attributed to a deficiency of a new blood clotting factor which these authors named plasma thromboplastin antecedent (PTA); it was present in serum, normal plasma, and plasma adsorbed by BaSO₄ precipitated by 25–33 per cent (NH₄)₂ SO₄ and found in Cohn fractions IV-I and III. The International Committee on the Blood Clotting Factors has recently reviewed the properties of PTA and assigned it the designation, factor XI.

PTA acts early in the sequence of blood coagulation as observed in the test tube. On exposure of blood or plasma to an appropriate surface the Hageman factor (HF, factor XII) is adsorbed and activated. Activated Hageman factor then changes PTA from an inert precursor to an active form, probably synonymous with what Waaler terms the activation product. These reactions can occur in the absence of calcium; in the presence of ionic calcium activation product initiates a series of reactions involving factors IX, VIII, X, platelet factor 3 and factor V and leading to the formation of the complete prothrombin converting activity, blood thromboplastin. Waaler, Margolis and Soulier and Prou-Wartelle have each shown that in the absence of Ca++, the activation product is gradually and irreversibly inactivated in plasma at 37 C. Since normal plasma contains an excess of HF as compared to PTA, it should be possible to find a concentration of activating material which will permit activation and exhaustion of PTA without completely removing HF.

The present study applies Waaler's method for exhausting normal plasma of activation product to the assay of PTA deficiency.

MATERIAL AND METHODS

Blood samples were collected using siliconized needles and syringes. When large volumes were required, blood was collected by gravity drainage through a blood collection set (Abbott Laboratories, North Chicago, Ill.). In each case the first 5 ml. was discarded to exclude contaminating tissue juice. One-tenth volume of 3.8 per cent sodium citrate was used as an anticoagulant for the preparation of plasma. Glassware and pipettes were coated with silicone (Z-4141, Dow Corning Corp., Midland, Mich.) or new previously unused glassware was employed. Imidazole-buffered saline solution, pH 7.3, was used for dilutions. Human brain cephalin was made by the method of Bell and Alton. Diatomaceous silica (Filter-cel) was obtained from Johns-Manville, Lumpoc, Calif.

Plasma was artificially depleted of PTA by the following modification of Waaler's method: platelet-poor plasma, centrifuged at 39,000 g for 30 minutes, was shaken with 15 mg. per ml. of Filter-cel powder for 5 minutes. The powder was removed by centrifugation at
Fig. 1.—Curve obtained when dilutions of five pooled normal siliconized plasmas were tested for PTA content with exhausted plasma. A 1:5 dilution is considered as the 100 per cent value.

39,000 g for 30 minutes. The supernatant plasma was adjusted to pH 7.0 with 0.1 N HCl or NaOH and incubated for 6 hours at 37 C. with frequent mixing. Cephalin clotting time of the preparation gradually increased from approximately 80 seconds to over 150 seconds.

The PTA assay was performed by the method described by Waaler for measurement of activation product.‡ Test plasma was collected and freed of platelets in silicone-coated tubes; it was diluted 1:4 and 1:9 with buffer. One volume each of diluted test plasma, cephalin suspension and exhausted plasma were incubated for 6 minutes at 37 C. in new glass tubes, then recalciﬁed with a volume of 0.025 M CaCl2. The tube was kept in the water bath for a minute, then tilted in air till evidence of a clot was seen. Tests were performed in duplicate and the mean value taken when the results agreed within 10 seconds. The results were compared to dilution curves of plasma from five normal individuals done on the same batch of exhausted plasma (ﬁg. 1). The test curve was generally parallel to the normal dilution curve and the results were interpolated to give the per cent of normal PTA values. If test results did not agree within 10 seconds, or if the test curve did not parallel the normal curve, a fresh dilution was made and the test repeated.

PTA assays were also performed using as substrate fresh and frozen plasma from a patient severely depleted of this coagulant (Case 1 of this report). The method described by Rapaport et al.10 was employed. Frozen samples of plasma from our patients were also assayed through the courtesy of Dr. Robert L. Rosenthal.

Fibrinogen was measured by an ammonium sulfate turbidity procedure,11 the prothrombin complex by the one-stage prothrombin time and the test of Ware and Stragnell.12 Spaet’s modification was employed for the thromboplastin generation test except that citrated plasma was adsorbed with aluminum hydroxide to prepare the plasma reagent, and pooled normal citrated plasma was used for substrate. Factors V, VII, and X were individually assayed by a procedure similar to the Ware and Stragnell test, but using the following substrate plasmas: factor V- oxalated human plasma, incubated at 37 C. to inactivate factor V; then dialyzed against 0.38 per cent sodium citrate buffered with 0.01 M imidazole at pH 7.3 to obtain a stable reagent;14 factor VII-citrated plasma from patients recently treated with bishydroxycoumarin; factor X-congenitally deﬁcient plasma. Factor VIII was measured by the ability of Al(OH)3 adsorbed plasma to correct the defect of factor VIII deﬁciency in a modiﬁcation of the thromboplastin generation test. Factor IX and HF were assayed in a partial thromboplastin test system using congenitally deﬁcient plasma, test plasma diluted 1:4 in imidazole buffer, and cephalin; these results were read
Table 1.—Coagulation Values in Artificially Depleted Normal Plasma (Exhausted Plasma) and in Members of a Family with PTA Deficiency

<table>
<thead>
<tr>
<th>Test*</th>
<th>Exhausted Plasma</th>
<th>Case 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (mg. per 100 ml.)</td>
<td>210</td>
<td>190</td>
<td>150</td>
<td>200</td>
<td>400</td>
<td>276</td>
<td>200-400</td>
</tr>
<tr>
<td>Prothrombin time (sec.)</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>11-12</td>
</tr>
<tr>
<td>Prothrombin (Ware &amp; Strangell) (per cent)</td>
<td>113</td>
<td>72</td>
<td>86</td>
<td>70</td>
<td>71</td>
<td>70</td>
<td>70-120</td>
</tr>
<tr>
<td>Factor V (per cent)</td>
<td>76</td>
<td>70</td>
<td>74</td>
<td>70</td>
<td>74</td>
<td>76</td>
<td>70-120</td>
</tr>
<tr>
<td>Factor VII (per cent)</td>
<td>100</td>
<td>100</td>
<td>70</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Factor VIII (per cent)</td>
<td>100</td>
<td>100</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Factor IX (per cent)</td>
<td>100</td>
<td>100</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Factor X (per cent)</td>
<td>100</td>
<td>100</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Hageman factor (per cent)</td>
<td>87</td>
<td>190</td>
<td>130</td>
<td>105</td>
<td>88</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Serum prothrombin (per cent)</td>
<td>---</td>
<td>52</td>
<td>5</td>
<td>64</td>
<td>40</td>
<td>34</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>Thromboplastin generation†</td>
<td>Test plasma-normal serum (sec.)</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Normal plasma-test serum (sec.)</td>
<td>---</td>
<td>50</td>
<td>8</td>
<td>12</td>
<td>9</td>
<td>11</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Test plasma-test serum (sec.)</td>
<td>---</td>
<td>75</td>
<td>10</td>
<td>45</td>
<td>9</td>
<td>12</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Bleeding time (min.)</td>
<td>---</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2-7</td>
</tr>
<tr>
<td>Clotting time (min.)</td>
<td>---</td>
<td>25</td>
<td>20</td>
<td>25</td>
<td>16</td>
<td>20</td>
<td>5-16</td>
</tr>
<tr>
<td>Fibrinolysis (mm.)</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td>120</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td>&gt;120</td>
</tr>
</tbody>
</table>

PTA (Per Cent)

| Method 1 (11) | 0 | 1 | 50 | 0 | 50 | 0 | --- |
| Method 2 | 7 | 9 | 60 | 7 | 42 | 29 | --- |
| Exhausted plasma | --- | --- | 6 | 55 | 13 | 48 | 14 | --- |

*See text for methods.
†Minimum substrate time after 6 minutes of incubation.
§Plasma from this patient was used as substrate in the assay.
¶Assays performed on frozen plasma specimens by the kind courtesy of Dr. R. L. Rosenthal.

from dilution curves prepared from normal plasma. Fibrinolytic activity was estimated by the euglobulin method.15

RESULTS

Plasmas depleted of PTA by the method described here were found to contain normal percentages of fibrinogen, prothrombin complex, and factors V, VII, VIII, IX, X and HF (table 1). Waaler, using larger amounts of celite powder to activate and exhaust his plasmas, found normal amounts of all clotting factors except HF, which was depleted, while Soulier and Prou-Wartelle found that 15 mg. per ml. of celite powder treatment removed PTA without completely depleting HF.7 Use of this reagent as a specific assay for PTA was applied to five members of a family with PTA deficiency, described in the appendix. Results were compared to two methods using congenitally deficient plasma as a substrate and are presented in table 1. The striking agreement between the three methods indicates that under these circumstances the artificially depleted plasma accurately measures PTA levels.

That this test is specific for PTA deficiency is indicated in table 2 where a variety of congenital and acquired coagulation disorders are evaluated. Values below 70 per cent were found only in patients with congenital PTA deficiency and in patients with severe liver disease. Normal or elevated values were found in patients with factor VIII, IX and HF deficiency, Von Willi-
Table 2.—PTA Values in Normal Individuals and in Patients with Various Coagulation Deficiencies Using Artificially Depleted Normal Plasma Assay

<table>
<thead>
<tr>
<th>Subjects</th>
<th>#</th>
<th>Clotting Time* (sec.)</th>
<th>% PTA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>20</td>
<td>74–94</td>
<td>70–&gt;200</td>
</tr>
<tr>
<td>HF-deficient</td>
<td>1</td>
<td>91</td>
<td>83</td>
</tr>
<tr>
<td>Factor VIII-deficient</td>
<td>1</td>
<td>81</td>
<td>160</td>
</tr>
<tr>
<td>Factor IX-deficient</td>
<td>1</td>
<td>77</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Bishydroxycoumarin therapy</td>
<td>5</td>
<td>70–92</td>
<td>80–&gt;200</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>4</td>
<td>75–90</td>
<td>88–&gt;200</td>
</tr>
<tr>
<td>Von Willebrand’s disease</td>
<td>2</td>
<td>85–86</td>
<td>165</td>
</tr>
<tr>
<td>Liver disease</td>
<td>3</td>
<td>94–106</td>
<td>57–70</td>
</tr>
<tr>
<td>PTA-deficient</td>
<td>5</td>
<td>107–162</td>
<td>3–55</td>
</tr>
</tbody>
</table>

*Range of partial thromboplastin times given by the artificially depleted plasma test using 1:4 dilutions of test plasma.
†Obtained from normal dilution curve similar to figure 1.

brand’s disease, thrombocytopenia and on therapy with bishydroxycoumarin.

Our own experience with PTA assays employing plasma from patient 1 should be amplified. We have repeatedly obtained valid results using this material on the day of collection. All attempts to store this plasma in the frozen state have led to variable and unpredictable results, these despite careful attention to collection of blood, siliconizing surfaces, separating platelets, and mixing the plasma after thawing.16,17 The artificially depleted plasma, on the other hand, has given us reproducible and reliable results even after storage at −20 C. for 1 month.

An attempt was made to “improve” the naturally depleted plasma by subjecting it to the same exhaustion procedure as used for normal plasma.7 This preparation proved less useful than the artificially depleted normal plasma.

DISCUSSION

The method described in this report appears to satisfy the requirements for a reagent which is sensitive to deficiencies in PTA and can be used for the specific diagnosis of such deficiencies. When prepared using 15 mg. of Filter-ecel per ml. of plasma, exhausted plasma has been shown to contain normal amounts of other clotting factors. The reagent can be prepared by any laboratory investigating coagulation disorders independent of a source of congenitally deficient plasma. Results obtained using this reagent in an assay for PTA agree favorably with those using congenitally deficient plasma.

In agreement with the findings reported by Rapaport,16 we have found PTA values normal in patients anticoagulated with vitamin K antagonists and decreased in some patients with severe liver disease. Other congenital and acquired hemorrhagic disorders gave normal or elevated PTA values.

The precise diagnosis of PTA deficiency in the past has presented considerable difficulty. Since Rosenthal’s original description1 a number of similar cases have been reported.17,18–30 Laboratory studies have shown highly variable defects in the tests of the first stage of coagulation.3,17,18,36 Whole blood and
recalcified clotting time values have in some patients been slightly to moderately prolonged, both in glass and silicone tubes. Impaired prothrombin consumption (elevated serum prothrombin) and defects in thromboplastin generation tests have been noted with much greater frequency. The characteristic pattern observed in the thromboplastin generation test is an abnormal result when patient's adsorbed plasma and serum reagents are employed, corrected by addition of either normal BaSO₄-adsorbed plasma or normal serum reagent. Commonly, however, the correction with Al(OH)₃ plasma is only partial.³⁷ This pattern is not specific for PTA deficiency. It may be seen with HF deficiency or in the presence of circulating anticoagulant. On the other hand, in mild PTA deficiency the thromboplastin generation test may be completely normal.

The problem of precise identification of the PTA defect has been complicated by the finding that even the most severely affected cases possess some PTA activity³⁷ and that this activity tends to show an apparent increase when the patient's serum or plasma is stored in the lyophylized or frozen state. Thus material from PTA-deficient patients tends to become self-corrective on storage.

In only a few of the reported cases of PTA deficiency has the diagnosis been confirmed by the lack of correction of the patient's coagulation abnormalities by material from Rosenthal's original patients, and vice versa.²⁴,²⁶,²⁷,³³ Only a presumptive diagnosis has been possible in the remaining cases. The diagnostic problems presented are illustrated by Sjölin's survey of hemophilic diseases in Denmark.³⁶ Of 148 hemophilic patients, 15 were classified as PTA deficiency on the basis of the initial laboratory data. Further correction studies enabled the author to classify 10 of these patients as mild factor VIII deficiency and two others as mild factor IX deficiency; only three patients remained as probably PTA-deficient.

Aggeler⁴ has recently stated, "there is as yet no accurate method for asaying PTA" and "until an accurate method for PTA is available some doubt must be entertained as to the validity of the diagnosis when it is based solely on the results of the thromboplastin generation test."

Unlike classical hemophilia, the severity of the disease has varied widely among afflicted members of the same family. Rosenthal¹-³ originally postulated an autosomal dominant hereditary pattern with incomplete expression in the transmission of this disorder, and subsequent family studies have been interpreted as being in accord with this hypothesis.¹⁹,²⁴,²⁸,³¹,³⁵,³⁶ Rapaport and his co-workers¹⁷ have recently reported the largest study of PTA deficiency. Using an assay based on naturally occurring PTA-deficient plasma, they found that they could separate patients, their families and normals into three distinct groups:

1. severely PTA-deficient, with 3 to 20 per cent of normal activity;
2. mildly PTA-deficient, with 33 to 60 per cent of normal activity; and
3. normals, with greater than 67 per cent of normal activity.

Examination of pedigrees revealed that both parents of severely deficient patients were themselves deficient, but usually only mildly, whereas children of marriages between a severely deficient and normal parent invariably were
mildly PTA-deficient. These data were interpreted as indicating that the disorder is inherited as an incompletely recessive trait, with two degrees of deficiency recognizable. The severe defect—major PTA deficiency—results from the homozygous state with respect to PTA, while minor PTA results from the heterozygous state. In this experience a good correlation existed between PTA levels and clinical symptoms. Patients with minor PTA rarely bled from surgery and other trauma and did not usually consider themselves as bleeders, whereas those with major PTA deficiency had more serious and more frequent hemorrhages.

The exhausted plasma test should facilitate recognition of PTA heterozygotes and homozygotes. The family described in the appendix of this report can best be interpreted in the light of Rapaport's hypothesis.

**Summary**

Plasma artificially depleted of PTA by incubation following activation with diatomaceous silica was evaluated as a reagent for the assay of PTA. It gave results which were comparable to those obtained with naturally deficient plasma and had the advantages of being readily available and more stable on storage. Laboratory differentiation was possible between PTA and HF deficiency.

**AppenDIX**

**Patient Material**

Coagulation studies were done on five affected members of a family with PTA deficiency. The parents are both of Jewish extraction but there was no consanguinity. Figure 2 depicts the family relationship.

**Case 1.** D. W., the propositus, is a 19 year old male who bled so profusely from circumcision at 7 days of age that blood transfusions were needed to control the bleeding. A tonsillectomy at age 4 years induced bleeding which persisted for 3 weeks and was finally controlled with blood and plasma transfusions. His appendix was removed at age 11 and dental extractions were performed at ages 12, 16 and 17. Each procedure followed transfusion of fresh frozen plasma and was not complicated by excessive bleeding. The patient bruises easily and has frequent epistaxis, but no other spontaneous hemorrhages. Coagulation studies are characterized by a prolonged coagulation time, markedly impaired prothrombin consumption and thromboplastin generation, and between 4 and 18 per cent PTA by specific assay (table 1).

**Case 2.** B. W., brother of Case 1, is 16 years old. Because of their experience with his older brother, his parents delayed his circumcision until he was 4 months old; there was no excessive bleeding. Tonsillectomy at 3 years of age, however, was followed by hemor-
rhage and treated with plasma. Appendectomy and dental extractions at ages 11 and 16 were also successfully treated with prophylactic plasma transfusions. He notes easy bruising and frequent bleeding from the gums. On coagulation survey, a slight defect in thromboplastin generation and a prolonged clotting time are the only defects noted, and his PTA level is 54 to 66 per cent of normal (table 1).

Case 3. L. W. is the 10 year old sister of the above patients. She had an umbilical hernia repair at 9 months of age without hemorrhagic complications. Dental extractions have been prophylactically treated with plasma transfusions, and her recurrent tonsillitis treated by irradiation. The patient bruises readily and notes frequent nose bleeds. Her coagulation studies are similar to those of her more severely affected older brother and her PTA level is 7 to 13 per cent of normal (table 1).

Case 4. R. W. is the 40 year old mother of the above children. Tonsillectomy at 12 years of age resulted in hemorrhage requiring transfusions, as have several dental extractions. However, she underwent appendectomy at age 14 without complication. Her menses have always been heavy and hemorrhages have followed each delivery, necessitating blood transfusion each time. A thyroid adenoma was resected in January 1962 with plasma given to prevent hemorrhage. No bleeding complications were noted but 4 weeks later she developed hepatitis at which time profuse vaginal bleeding occurred. She has always bled and bruised easily and considers herself to be a bleeder. Her father likewise bled excessively, and died with refractory anemia in 1956. Her mother and other members of her family did not bleed overmuch. Coagulation studies and PTA assay on her mother were normal. The patient has persistently shown a slightly prolonged coagulation time, poor prothrombin consumption, and a mild thromboplastin generation defect. Her PTA level is 39-55 per cent of normal (table 1).

Case 5. N. W., the 42 year old father of Case 1-3 and husband of Case 4, did not consider himself to be a bleeder and gave no history of hemorrhagic disorders in this family. Unfortunately, members of this family were not available for study. He had a bilateral inguinal hernia repair and appendectomy at 7 months of age without bleeding in excess. Dental extraction at age 32 was followed by oozing for 24 hours but was brought under control by local measures. Because of our experience with other members of his family and the genetic data reported by Rapaport et al. suggesting that he might be afflicted with PTA deficiency, coagulation studies were done. They indicated a moderately severe coagulation defect with between 10 and 20 per cent of normal PTA activity (table 1). Dental extractions were then performed in the hospital without plasma coverage, but with close supervision. The wound oozed for 24 hours, then stopped, but bleeding recurred 10 days after the extraction and did not stop until plasma transfusion had been administered. In

Fig. 2.—Family tree with PTA deficiency found in both parents and all three children. The arrow indicates the propositus.
August 1962 the patient developed a bleeding peptic ulcer which has healed on medical management without transfusion therapy.

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ASSAY OF PTA


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