Hemorrhagic Disease with Circulating Inhibitors of Blood Clotting: Anti-AHF and Anti-PTC in Eight Cases

By JESSICA H. LEWIS, JOHN H. FERGUSON AND TULIO AREND

The association of hemorrhagic disorders with circulating inhibitors of blood clotting is relatively rare. A number of reports during the past 15 years, however, indicate that such problems can be clinically significant and that the physiologic understanding of their types and modes of action is worthy of continued investigation.

No complete classification of these various inhibitors is possible at this time as their precise nature and the ways they act have not been worked out in many instances. Theoretically, an inhibitor might be expected to act against one or more of the numerous clotting factors, namely, AHF, PTC, PTA, Spaet's "4th factor," (pro)thrombin, (pro)convertin, (pro)accelerin, fibrinogen, platelet factors, or tissue thromboplastin. In a recent panel discussion of the inhibitor problems, the following, although not all yet identified as "circulating" anticoagulants, were mentioned: heparin, heparin co-factor, antithrombin, lipid antithromboplastin, species-specific thromboplastin inhibitor, antiproconvertin (etc.), abnormal globulins, protease (fibrinolysin) destructive to certain clotting factors, and perhaps some antiproteases, besides specific inhibitors of AHF and PTC, which are especially relevant to the present studies.

A survey of the literature pertinent to this study stresses that the etiology may involve some type of immunoresponsse, exhibiting demonstrable analogies to other antigen-antibody reactions and often following some provoking incident, such as transfusion, pregnancy, allergy to antibiotic drugs, or occurring concomitantly with some profound disturbance of protein metabolism such as collagen disease, generalized lymphadenopathy, or rarely neoplasia, etc. About half the reported cases are in patients already afflicted with a severe coagulation deficiency, e.g., hemophilia, PTC-deficiency, and often in these cases transfusion seems to be the provoking incident. The remaining cases acquire an inhibitor de novo, and the term "idiopathic" covers real ignorance as to the cause, although any of the concomitants above mentioned may be recorded for the individual case.

The present study resulted from the laboratory examination, with a battery of tests designed to shed light upon individual factors in the clotting and hemostatic mechanisms, of 240 patients with bleeding tendencies and another 160 without. In 8 cases, namely, 5 out of 52 hemophilies, 2 out of 26 PTC-deficients, and in one other, the presence of circulating inhibitors was demonstrated and special tests showed them to have anti-AHF or anti-PTC activities.

From the Department of Physiology, University of North Carolina, Chapel Hill, and the Department of Medicine, Duke University.

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CASE REPORTS

PTC Deficiency

R. J., #5, a 16 year old colored boy was admitted to Duke Hospital in February 1952 for diagnosis and treatment of a bleeding tendency. There was no family history of excessive bleeding. The patient showed normal development and no excessive bleeding until 3, when he bled extensively from a tooth socket. From age 6 to 16 he suffered frequent hemorrhagic episodes, usually following trauma, and including bleeding from cuts, hematomata and hemarthroses. During the year before admission he suffered repeated injuries to the left knee. Two months before admission this knee was very large, hot and painful. When tapped by his doctor it was found to contain infected bloody fluid and penicillin was instilled. After the third tap the patient bled profusely from the needle puncture and received 7 transfusions in the 3 weeks before admission.

Physical examination showed a poorly developed, slightly obese boy, complaining of pain in the left knee. Examination of the chest and abdomen was negative. The genitalia were under-developed. The left elbow was moderately stiff. The left knee was very large and painful. Bloody material drained from previous puncture wounds.

 Shortly after admission to the hospital, a coagulation time of 105 minutes (at room temperature) was recorded. The patient received 300 ml. of frozen-fresh plasma with only a slight drop in the coagulation time to 58 minutes. On the next day, his coagulation time was recorded as 2½ hours and he received 500 ml. of whole blood. Shortly after this transfusion, his coagulation time was recorded as 12 minutes. Two days later, he received one liter of whole blood (fresh), but the clotting time remained over one hour. Again, two days later, he received 2 units of blood without lowering of the clotting time. Toluidine blue was also administered without effect on the clotting time. Our first laboratory studies were carried out one week after admission and have been extended on a number of occasions. Antibiotic therapy cleared up the knee infection and the anticoagulant (see below) gradually disappeared. Studies at this time revealed an underlying PTC deficiency. Subsequently one severe bleeding episode led to a transfusion in another hospital (1953) followed by reappearance and gradual disappearance of the anticoagulant.

W. B., #224, an 11 year old white boy was tested because of a long standing bleeding tendency. His family history revealed a number of male bleeders on the maternal side, two of whom, an uncle W. S., #133 and a brother D. B., #223, proved to have PTC deficiency without detectable anticoagulant. W. B. gave a history of easy bruising and epistaxis during infancy and frequent hemarthroses, hematomata and hematuria since the age of 3. He had received numerous transfusions of whole blood and of plasma, with apparent benefit. The last transfusion was 3 months before the current examination. It should be noted that the severity of his bleeding tendency and frequency of transfusions did not differ significantly from those of his brother and uncle.

Hemophilia

Detailed case histories will not be given on the 5 (#162, 12, 18, 174 and 231) hemophiliacs. Family histories were positive in each case. All had suffered from the usual hemarthroses, hematomata and post traumatic bleeding. All had received many transfusions of whole blood, fresh and fresh-frozen plasma. Four of the patients were referred for clotting tests while the fifth, W. C., #162, was first seen after clinical failure to respond to transfusions of plasma and fresh blood. In preparation for a tooth extraction he had received 200 ml. of fresh-frozen plasma and hospital tests showed a satisfactory clotting time. On the second day after the extraction, however, he developed a large submandibular hematoma which dissected down the neck, necessitating tracheotomy. Further extension and shock symptoms occurred despite every effort of treatment. In the 18 days before death he received 400 transfusions, 232 being fresh whole blood (plastic bag) and 168 plasma. He also received hydrocortisone for a week and a few units of “human fibrinogen (dried)”, supplied through the courtesy of the American Red Cross. Platelet count varied from 173,000 to 350,000 despite the numerous transfusions.
Idiopathic

L. T., #6 was a 37 year old white housewife admitted to Duke Hospital in 1952 for diagnosis of a bleeding tendency. Family history was negative for bleeding disorders. She had been in good health without any bleeding difficulties during the first 22 years of her life. Then, following an uneventful pregnancy and delivery, her first three menstrual periods were very excessive and required whole blood transfusions on 5 occasions. For the next 12 years she disclaims any bleeding symptoms, although her husband stated that she did bruise easily and bled readily from minor trauma during this period. In 1949 she was hospitalized with the complaint of left upper quadrant abdominal pain and the presence of anemia and splenomegaly were reported. She received 15 units of blood during the next 6 weeks, and, for about 6 months thereafter noted menorrhagia, easy bruising and bleeding from the gums which gradually stopped. For the next two years she was free of symptoms. In 1951 her menses stopped abruptly and have not recurred. About a year before admission, following a mosquito bite, she developed a large painful blue swelling over the sternum. On hospitalization she was found to be anemic and was transfused. Shortly after the first of these 15 transfusions she suffered a 2 week period of moderate painless jaundice. Easy bruising and gum bleedings reappeared. Later in 1951 a large bluish painful swelling of the left upper arm appeared spontaneously. Re-hospitalized, this area ruptured and pulsatile bleeding was stopped by the surgical application of Gelfoam. Drainage of sanguineous purulent fluid continued, however, and complete pressure paralysis of the arm developed. With failure of repeated transfusions to effect improvement, she was transferred to Duke Hospital.

On physical examination she appeared a well developed and well nourished woman with pale yellowish skin showing some areas of pigmentation and vitiligo over the sternum and left arm. Numerous ecchymotic areas were widely distributed over the entire body. The abdomen was distended and shifting dullness and a fluid wave elicited. The liver and spleen were moderately enlarged. The upper left arm was swollen and blue with a draining sinus in the left axilla. Flaccid paralysis of the left arm and muscular weakness of both lower extremities with foot-drop were described.

Laboratory examination showed a hemoglobin of 7.9 Gm. per cent, a normal white count, evidence of normoblastic hyperplasia, a thymol turbidity of 11 units with 4+ flocculation, serum albumin of 4.2 Gm. per cent, globulin 5.8 Gm. per cent and stool urobilinogen of 365 Ehrlich units per day. Cortisone therapy, 300 mg. daily for one week, failed to improve her clinical condition or anticoagulant titer. She left the hospital after three weeks and no further follow-up has been possible, but her demise has been reported.

METHODS

Most of the laboratory methods employed in this study have been described.7-11 Special tests for inhibitors were carried out on all patients with prolonged clotting times or markedly defective prothrombin consumption.

Detection of Circulating Anticoagulant. Whole blood method. Blood drawn simultaneously from the patient and a normal subject, was distributed in the amounts shown in table 3, mixed gently and clotting times determined at 37 C. Plasma method. The following mixtures of oxalated plasma were prepared in new 12 x 75 mm. tubes, recalcified with 0.2 ml. 0.025 M CaCl2 and the clotting times recorded at 37 C.

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's plasma ml.</td>
<td>0.00</td>
<td>0.05</td>
<td>0.10</td>
<td>0.15</td>
<td>0.20</td>
<td>0.25</td>
</tr>
<tr>
<td>Normal plasma ml.</td>
<td>0.25</td>
<td>0.20</td>
<td>0.15</td>
<td>0.10</td>
<td>0.05</td>
<td>0.00</td>
</tr>
</tbody>
</table>

After 1 hour the clot was wound out and prothrombin assayed in the serum. A prolonged clotting time or less than 90 per cent prothrombin consumption in tubes 2-5 was considered positive. If tube 2 was positive, it was repeated using dilutions of the patient's plasma and the titer reported as the highest dilution still able to reduce the prothrombin consumption below 90 per cent.

Detection of Anti-AHF. Equal volumes (0.1 ml.) of normal plasma (diluted 1-5) and patient's plasma or saline were incubated at room temperature for 30 minutes to permit full
action of any inhibitor on the AHF in the normal plasma. Thereafter, 0.05 ml. of this mixture was added to 0.2 ml. of standard hemophilic plasma and assayed for AHF content in the usual manner. If incubation with the patient’s plasma had reduced the normal AHF content, the test was repeated employing dilutions of the patient’s plasma and the highest dilution causing loss of AHF activity reported as the titer.

Detection of Anti-PTC. This was performed in the same manner as the preceding, but the incubated mixtures were assayed for PTC content, employing a standard PTC deficient plasma as the substrate.

Preparation of AHF Fraction: One ml. cold absolute alcohol was added to 5 ml. cold oxalated plasma dropwise with constant stirring. The mixture was chilled for 20 minutes in an ice bath, centrifuged for 10 minutes at 2500 r.p.m., and the precipitate dissolved in saline to 5 ml.

Preparation of PTC Fraction: 500 mg. barium sulfate was mixed with 5 ml. oxalated plasma, the barium sulfate separated by centrifugation, washed once with saline, and eluted with 1 ml. 0.2 M sodium citrate. When this fraction was tested care was taken to use equal citrate concentration in dilutions and in the control and to use optimal calcium in recalcification.

RESULTS

Table 1 presents the abnormal results obtained in our usual test in these 8 patients. Note the prolonged clotting-times and failure of prothrombin consumption. Assays for AHF and PTC on the plasmas of patients L. T. and R. J. were low. When these assays were repeated on plasma fractions (*in table) and compared to normal fractions, similarly prepared, L. T. showed normal levels of AHF and PTC and R. J. showed normal AHF but not PTC. Additional tests on the fractions showed both PTCs and R. J.’s AHF to be free of anticoagulant properties. L. T.’s AHF showed a slight trace of anticoagulant. The other AHF and PTC assays were carried out on plasma.

Other tests: bleeding time, tourniquet test, platelet count, platelet thromboplastin, platelet accelerator, serum serotonin (on R. J., W. B., W. T., H. R., D. J., J. K.), clot retraction, prothrombin time, prothrombin, proconvertin, proaccelerin and fibrinogen, were normal in all except D. J., a 6 month old boy, who showed 50 % PTC, 64 % prothrombin and 49 % proconvertin, and L. T. who had a 56 % proconvertin level.

Table 2 presents data concerning inhibitors in these patients. The presence
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Table 2.—Tests for Inhibitors

<table>
<thead>
<tr>
<th>Name</th>
<th>Anticoagulant</th>
<th>Anti-AHF</th>
<th>Anti-PTC</th>
<th>Anti-Thrombin</th>
<th>Anti-tipl</th>
<th>Prothrombin Titration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. J.</td>
<td>0</td>
<td>+ (1:16)</td>
<td>+ (1:32)</td>
<td>+ (1:32)</td>
<td>+ 0</td>
<td>0</td>
</tr>
<tr>
<td>W. B.</td>
<td>0</td>
<td>+ (1:1)</td>
<td>0</td>
<td>+ (1:8)</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>W. C.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>W. T.</td>
<td>0</td>
<td>+ (1:16)</td>
<td>0</td>
<td>0</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>H. R.</td>
<td>0</td>
<td>0</td>
<td>+ (1:2)</td>
<td>0</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>D. J.</td>
<td>0</td>
<td>0</td>
<td>+ (1:1)</td>
<td>0</td>
<td>+ 0</td>
<td>0</td>
</tr>
<tr>
<td>J. K.</td>
<td>0</td>
<td>0</td>
<td>+ (1:1)</td>
<td>0</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>L. T.</td>
<td>+</td>
<td>+ (1:256)</td>
<td>+ (1:1024)</td>
<td>+ (1:256)</td>
<td>+ 0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3.—Clotting Times (Minutes) of Whole Blood Mixtures (37 °C.)

<table>
<thead>
<tr>
<th>Normal Blood (ml.)</th>
<th>R. J.'s Blood (ml.)</th>
<th>2.0</th>
<th>1.0</th>
<th>1.5</th>
<th>1.5</th>
<th>1.0</th>
<th>0.5</th>
<th>0.0</th>
<th>0.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/29/52</td>
<td>7</td>
<td>61/2</td>
<td>61/2</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>&gt;120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/3/52</td>
<td>61/2</td>
<td>161/2</td>
<td>24</td>
<td>84</td>
<td>&gt;120</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of a circulating anticoagulant has been demonstrated by observing clotting times of mixtures of whole blood from normals and patients. This method has proved unreliable in our hands in spite of simultaneous vein punctures, siliconized syringes, and careful mixing. Table 3 illustrates two such tests on patient R. J. On the first date no anticoagulant was demonstrable, while four days later potent inhibitor effect was apparent. Plasma anticoagulant titers (see figure 1) were positive on both dates.

Plasma anticoagulant titers on R. J. are illustrated in figure 1. Similar mixtures were prepared for all patients and were considered positive if any tube, containing both normal and patient's plasma, showed a prolonged clotting time (>5 min.) and an abnormal prothrombin consumption (<90%). If the second tube (0.2 ml. normal + 0.05 ml. patient) was positive the patient's plasma was diluted and the greatest dilution showing anticoagulant effect reported as the titer (table 2). Figure 1 illustrates the fall in anticoagulant in R. J. during a six month period in which transfusions were avoided. Subsequently this patient received another transfusion and again his plasma showed marked anticoagulant properties which gradually disappeared over the next year.

Anti-AHF and Anti-PTC were demonstrated by incubating the patient's plasma with normal plasma and then titering the AHF or PTC content of the mixture. When ordinary hemophilic or PTC deficient plasma was incubated with normal plasma no decrease in the AHF or PTC content of the normal plasma was demonstrable. In these patients a marked drop in AHF or PTC occurred. Even in great dilution the plasma of some of the patients was inhibitory.

Antithrombin in the plasma was sought by comparing the clotting times of patient's plasmas with normal plasma after addition of the same amount of thrombin. The thrombin concentration was adjusted so that the normal clotting time was 15 ± 2 secs. Patient's clotting times were considered prolonged if they were greater than 2 sec. longer than the normal. Such prolongations (4 to 7
secess.) were observed in L. T., R. J., and D. J. Serum antithrombin titers were unsatisfactory, as in every case, the serum (1 hour from venipuncture) contained some fibrinogen.

An inhibitor to human tissue thromboplastin (Anti-thpl) was sought by substituting human brain for ordinary thromboplastin in a prothrombin time test. None of the patients showed any indication that their inhibitors were antithromboplastic.

In a search for heparin or heparin-like effects, protamine titrations were carried out in most instances. In no case did any concentration of protamine sulfate shorten the recalcification time.

Cases R. J. and L. T. were subjected to the following additional studies:

1. Electrophoretic studies showed significant elevations in gamma globulin. Figure 2 compares a normal, L. T. (#1), and R. J. (#2), when his anticoagulant titer was high and, again, when it had fallen. The gamma globulin concentrations calculated as per cent of total protein were normal = 12%, L. T. = 27%, R. J. (3-4-52) = 35% and (7-8-52) = 18%.

2. Fractionation Studies: Adsorption with barium sulfate, kaolin or glass wool, dialysis against saline, and treatment with ether, chloroform, acetone (20%) or ethanol (20%) did not remove the anticoagulant.

A series of fractions were prepared by adding increasing concentrations of saturated ammonium sulfate. The precipitates collected between 25 and 33 per cent saturation contained all of the anticoagulant from R. J.'s plasma and most of that in L. T.'s.

3. Stability. Anticoagulant properties of both L. T. and R. J.'s plasma were stable at 56 C. for 5 min. and at −20 C. for four years. Both were destroyed at 75 C. for 5 min.
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4) Attempts to isolate Tocantin's lipid antithromboplastin\(^ {23} \) from both plasmas were unsuccessful.

5) Both plasmas were set up for precipitin reactions\(^ {3} \) using an AHF containing Cohn fraction I as the antigen. No precipitins were demonstrated.

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**Fig. 2.**—Electrophoretic analysis of plasmas. Case 1: idiopathic circulating anticoagulant, Case 2: PTC deficient with anti-PTC (3-4-32) and without (7-8-32).

**DISCUSSION**

A whole blood mixture method is commonly used for demonstrating the presence of an anticoagulant. Such a test proved positive on L. T. on two occasions and R. J. on one occasion. On another date R. J.'s test was negative although a plasma-mixture anticoagulant test was positive. In the plasma method only a few seconds are allowed to elapse between preparation of the mixture and recalcification. None of the hemophilies gave positive results, and, even in the mixture containing 0.2 ml. patient's plasma and 0.05 ml. normal plasma the clotting
times and prothrombin consumptions were normal. In spite of this lack of anticoagulant action it was possible to demonstrate anti-AHF activity by allowing normal plasma and the patient’s plasma to preincubate before testing for residual AHF activity. In case W. C. it was possible to show an increasing loss of AHF during the first few minutes that his plasma was incubated with normal plasma indicating that this inhibitor must have sufficient reaction time in order to demonstrate its presence.

The anticoagulant activity of the two PTC deficient plasmas clearly seemed due to a specific inhibitor to PTC. In R. J., with the more potent inhibitor, an apparent slight anti-AHF effect was noted. This may be due to an inhibition of PTC in the AHF substrate and would not be unexpected considering the ability of this patient’s plasma to prolong normal clotting.

L. T., whose plasma showed very marked anticoagulant activity, also showed both anti-AHF and anti-PTC. As the anti-AHF was the more potent, it is again possible that the anti-PTC effect was simply due to inhibition of AHF in the PTC substrate.

The plasma-antithrombin tests (plasma-thrombin clotting times) were of doubtful assistance in determining the site of action of these anticoagulants. Such prolongations also occur in hypofibrinogenemia, new borns, hyperheparinemia and in some leukemias and other disorders. In patient D. J. this may relate to his infancy, as probably also do the reductions in prothrombin, proconvertin and PTC.

The foregoing results indicate the existence of two specific inhibitors, anti-AHF and anti-PTC, typically appearing in severe cases of the corresponding deficiency diseases, probably as an unusual isoimmunization resulting from transfusion therapy. The idiopathic case did not lack the plasmatic factors, but showed very powerful inhibitors to both, the anti-AHF predominantly. It is difficult to date the onset of her bleeding tendency. Either a pregnancy 14 years earlier or the many subsequent transfusions might be involved.

Lack of response to blood transfusion may suggest the presence of an inhibitor, although 5 of our patients had not noted this nor had they shown any marked increase in clinical symptomatology. Once an inhibitor has been demonstrated, avoidance of future transfusions would seem indicated, particularly in view of the reappearance of anticoagulant in R. J.’s plasma after a small transfusion. We do not have sufficient data to determine how long such inhibitors persist. R. J.’s titer fell over 6 months to 1 year on two occasions. H. R. showed anti-AHF on three examinations over a three year period during which he had received approximately 4 units of plasma per year.

No indications of why an inhibitor develops were obtained. D. B. and W. C. each had brothers without demonstrable inhibitors but with similar deficiencies, each of whom had received about the same number of transfusions, either of whole blood or plasma (fresh or frozen). All of the patients who showed inhibitors were severe bleeders, apparently completely lacking in the primary factor.

**Summary**

In eight patients suffering from severe hemorrhagic disease it was possible to demonstrate the presence of circulating inhibitors, not heparin-like in nature.
Anti-PTC was identified in two PTC deficient, anti-AHF in 5 hemophilies and both, primarily anti-AHF, in one idiopathic case. Possible etiologic factors inducing the appearance of such inhibitors included repeated transfusions of whole blood and plasma in all and a previous pregnancy in the idiopathic case. Two patients showed increases of gamma-globulin and presence of inhibitor in 25–33 per cent ammonium sulfate plasma fractions. Cortisone therapy was ineffective in two cases.

**SUMMARIO IN INTERLINGUA**

In octo patientes sufrrente de un sever morbo hemorrhagic il eseva possibile demonstrar le presentia de inhibitores circulante, que non eseva de natura simile a heparina. Le inhibitor anti-PTC eseva identificate in duo patientes deficient in PTC; anti-AHF eseva identificate in 5 hemophiliacos e ambe, primarimente anti-AHF, in un caso idiopathic. Le possibile factores etiologic que induceva le apparition de iste inhibitores includeva in omne casos repetite transfusiones de sanguine integre e de plasma, e in le caso idiopathic un previe pregnantia. Duo patientes monstrava accrescite globulina gamma e le presentia del inhibitor in fractiones del plasma preparate con sulfato de ammonium in concentrationes de inter 25 e 33 pro cento. Therapia a cortisona eseva inefficace in duo casos.

**REFERENCES**

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