CASE REPORT

A Coagulation Defect due to an Anticoagulant Possessing Antithromboplastic and Antithrombic Properties, Probably Heparin

By Warren N. Bell, M.D.

The demonstration that an excess of heparin or of heparinoid substances in the blood may occasionally be responsible for the development of a hemorrhagic disorder has recently assumed some practical importance because of the availability of specific antidotes, namely, protamine sulfate and toluidine blue. A prolonged coagulation time due to an excess of the heparin factor shows a specific therapeutic response, thus differing from the response in hemophilia, afibrinogenemia and hypoprothrombinemia which are likewise characterized by a prolonged coagulation time. The present case report deals with a patient with excess heparinoid substances in his blood who showed a specific response to protamine sulfate therapy.

Case Report

P. V., a 42 year old silk weaver of Italian origin, was first seen November 14, 1950. At the age of 7 he had prolonged bleeding following each of four tooth extractions. At 17 he fractured his nasal septum and had daily bouts of epistaxis for more than a month. A subsequent tonsillectomy required suturing of the pillars to stop severe postoperative bleeding. Superficial small cuts did not bleed excessively. At 37 he began to have spontaneous subcutaneous and intramuscular hematomata, usually in the extremities or about the umbilicus. At 39 he had a bout of hematuria. At the time of his admission he complained of recurrent hematuria and frequent painful subcutaneous and intramuscular hematomata.

He was diagnosed as having hemophilia in 1945 and received several transfusions of fresh blood and plasma without benefit. In 1947 the diagnosis of "hypoprothrombinemia" was made but injections of plasma and vitamin K failed to stop the bleeding. Liver, rutan, vitamin C and "thrombin" injections were given at intervals, also without benefit.

The patient related a rather vague story of a male cousin having "bled to death in bed" several years before but gave no other pertinent family history.

Physical exam was not remarkable except for two fading hematomata of the right thigh. Hemoglobin was 13.8 Gm., white blood cells numbered 6,500 and the urine was free of hemoglobin. Thymol turbidity and flocculation tests, cephalin cholesterol flocculation, bromsulfalein and bilirubin determinations were all within normal limits.

Investigation of the Coagulation Defect

1. Bleeding time (Duke): 4.5 minutes but the wound began to bleed again twenty-four hours later. 2. Clotting time (Lee-White): 105 to 110 minutes with good clot retraction (normal, 12 to 18 minutes). 3. Platelets: 175,000 to 225,000, Illes and Ecker method on venous blood (normal, 175,000 to 300,000). 4. Platelet adhesive index (method of Moolten and Vroman): 2.44 (normal, 1.0 to 1.4) with 112,000 adhesive platelets (normal, 60,000 to 110,000). 5. One stage prothrombin time (Quick): 53.0 to 55.2 seconds using human brain thromboplastin (normal, 13.0 seconds). 6. Prothrombin Consumption Test: 17 per cent in

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1 hour, 58 per cent in 3 hours and 95 per cent in 24 hours using the modified one stage test. When this test was repeated using the two stage method there was 60 per cent consumption in 1 hour (normal, 65 to 90 per cent consumption in 1 hour). 7. Modified two stage prothrombin test (Ware and Seegers\(^3\)): 255 units of prothrombin (normal control, 270 units).

8. Modified two stage prothrombin test (Ware and Seegers\(^3\)): 255 units of prothrombin (normal control, 270 units).

9. When the two stage test was repeated without using beef serum as a source of Ac-globulin, no difference was noted, suggesting that adequate Ac-globulin was present.

10. Clotting time of recalcified oxalated plasma which had been centrifuged at 1000 r.p.m. for 5 minutes was 250 seconds (normal, 90 to 120 seconds).

11. Fibrinogen: 640 mg. per cent (normal, 350-700 mg).

12. Antithrombin activity: 500 units of purified thrombin (Parke-Davis) were added to each of two test tubes containing 1 cc. of plasma (normal control and patient) and the thrombin remaining in the serum was titrated in both cases after one hour. The control showed 300 units and the patient 180 units. Thus it appeared that the patient's plasma was definitely exerting an antithrombin effect. However, since Klein and Seegers\(^7\) have shown that considerable thrombin is adsorbed on the fibrin, we then repeated the test adding just sufficient 60 per cent urea solution to both tubes to inhibit fibrin formation (this approach was used instead of dissolving the fibrin clot as recommended by these authors due to the unavailability of streptolysin). We have been able to show in other studies (unpublished data) that 60 per cent urea has no detrimental effect on thrombin activity after 24 hours at 25 C. nor does it interfere with the end-point in the thrombin titration. On this occasion the control showed 130 units of thrombin remaining after 1 hour and the patient 90 units. Thus it would appear that most of the 'antithrombin' activity was due to an increase in the amount of thrombin adsorbed on the fibrin in the patient's plasma.

13. Circulating anticoagulant (table 1): the results of this test strongly suggest the presence of an anticoagulant in the patient's plasma which prolonged the clotting time of normal plasma when the two plasmas were mixed in varying proportions.

14. Antithromboplastic activity: The patient's plasma was tested for antithromboplastic activity by: (a) adding excess thromboplastin to both plasmas in the performance of the one stage prothrombin test (Quick) and then using the sera from both plasmas as the thromboplastin in performing a one stage test on the same normal plasma, the rationale being that any antithromboplastin which might be present would antagonize the excess thromboplastin so that the second one stage test should be even more prolonged. The results are presented in table 2. It was also tested by (b) incubating 0.5 cc. of each plasma with 0.15 Gm. thromboplastin for 2 hours and then using each to do a one stage prothrombin test on two samples of the same normal plasma. The control normal plasma time was 13.2 seconds while the time using the patient's plasma thromboplastin mixture on the normal plasma was 56.3 seconds. From the results of both these procedures it appears that the patient's plasma exerted a definite antithromboplastic activity.

Further tests were done to determine other characteristics of this anticoagulant. It was found to be maximally effective at 37 C., remained active after storage at 25 C. for 24 hours but was inactivated by heating at 50 C. for 5 minutes. The anticoagulant was removed.

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**Table 1—Test for Circulating Anticoagulant**

<table>
<thead>
<tr>
<th>Tube</th>
<th>NaCl 0.15M</th>
<th>CaCl(_2) 0.025M</th>
<th>Normal Plasma</th>
<th>Patient's Plasma</th>
<th>Clotting Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.00</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.05</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.10</td>
<td>12.5</td>
</tr>
<tr>
<td>4</td>
<td>0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.15</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.20</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.25</td>
<td>127</td>
</tr>
<tr>
<td>7</td>
<td>0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.30</td>
<td>142</td>
</tr>
<tr>
<td>8</td>
<td>0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.40</td>
<td>180(^*)</td>
</tr>
<tr>
<td>9</td>
<td>0.40</td>
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<td>180(^*)</td>
</tr>
</tbody>
</table>
or inactivated by deprothrombinization of the plasma using tri-calcium phosphate. The effect of the anticoagulant was nullified by diluting it 1:8 with saline.

14. **Heparin-like characteristics of the anticoagulant:** In a further effort to define the nature of this antithromboplastic substance, a protamine titration test was done and it was found that 0.18 mg. of protamine sulfate was required to clot 1 cc. of the patient’s blood in one hour, whereas a normal control blood required 0.14 mg. This difference is considered significant by Allen. A quantitative heparin-protamine titration test was then done and the results are listed in table 3.

The principle of the test outlined in table 3 is that the shortest clotting time is found in the tube where just sufficient protamine has been added to nullify both the exogeneous heparin which has been added and any endogeneous heparin or heparinoid substances which may be present in the patient’s plasma. Confirming LeRoy and his co-workers, we have found that protamine neutralizes heparin in the ratio of 1.5:1 so that the shortest clotting time is normally found in tube 4. From the results in table 3, it is seen that the shortest clotting time is found in tube 8. On the basis of these data, it can be calculated that this patient’s plasma contained approximately 43 gamma of heparin or heparinoid substances per cc. of plasma. Jaques has shown that, normally, plasma contains 1 gamma per cc.

Investigation was then undertaken of the in vivo effects of protamine sulfate and fresh plasma on the patient’s clotting time. Five hundred cc. of plasma less than 24 hours old had no effect on the clotting time (110 minutes before and 115 minutes after the infusion). One hundred mg. of protamine sulfate given intramuscularly in two 50 mg. doses two hours apart reduced the clotting time from 108 minutes to 65 minutes 2 hours after the second injection, the effect lasting for 3 hours. After a 200 mg. dose of protamine sulfate was given intravenously, the clotting time was reduced from 106 minutes to 43 minutes, and two hours later was 95 minutes.

At the time of his discharge, the patient had had no further hemorrhages. A two month follow-up revealed that no further hemorrhages had developed and that he had been working constantly since his discharge. This was considered encouraging as he had been almost unemployed for the previous five years because of his hemorrhagic manifestations. Accordingly, there has not yet developed an opportunity of judging the therapeutic effect of protamine sulfate in the presence of hemorrhage in this patient.
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INTERPRETATION

The coagulation mechanism may be said to comprise three stages: (1) the activation of thromboplastin, (2) the activation of prothrombin to thrombin and (3) the interaction of thrombin and fibrinogen to form fibrin. In our patient, despite the abnormal one stage prothrombin test, the two stage test demonstrated that the actual prothrombin concentration was normal and that there was no apparent deficiency of Ac-globulin. The fibrinogen was also normal so that the basic elements necessary for the final two stages appeared to be present in adequate amounts. The total number of platelets and their function appeared to be normal. The prothrombin consumption, using the one stage test, suggested a poor consumption but, with the two stage test, the consumption was closer to normal. This, besides suggesting adequate thromboplastinogen, appears to indicate deficiency of some factor other than prothrombin, which might affect the prothrombin consumption as measured by the one stage test. The major fault in this patient appeared to be either a defective activation of thromboplastin or the presence of an excess of antithromboplastin. The results of the tests favor the latter alternative. This may explain the prolonged one stage prothrombin time as well as the normal two stage test due to the 1:25 dilution involved in the latter procedure.

Thus it would seem that this anticoagulant functions principally as an antithromboplastin and, to some extent, as an antithrombin. The results of the protamine titration tests suggest that this anticoagulant is heparin or a heparinoid substance. The validity of this conclusion based on in vitro tests appears confirmed by the results of administration of protamine sulfate.

DISCUSSION

This case demonstrates abnormalities which have not been previously reported. The result of the prolonged one stage prothrombin time, without the aid of the two stage method, might well have justified classification of this patient's case as one of idiopathic hypoprothrombinemia. This raises the question whether some of the cases reported under this designation may not have been cases of hyperheparinemia. Klein and Seegers have shown that the antithrombin effect of heparin is exerted mainly by increasing the adsorption of thrombin on the fibrin. This study appears to confirm their results. Tocantins has stated that antithromboplastin may act either directly to neutralize thromboplastin or indirectly by competing with thromboplastin for prothrombin. The same type of action would apply to the anticoagulant under discussion. Our patient appears to differ from the case of acute antithromboplastinemia reported by Harrington and his associates in that their patient's anticoagulant was not adsorbed by tri-calcium phosphate and lost its activity after 2 hours at 37 C. There is no evidence to suggest that the anticoagulant in the patient reported here is similar to the anti-hemophilic globulin antibody which might have developed from previous transfusions of blood and plasma. In cases in which such antibodies have been demonstrated, they have been found to disappear spontaneously in six to twelve months. This patient had received no blood nor plasma for three years.

The clotting time of the recalcified oxalated plasma was definitely prolonged.
Quick has claimed that this is characteristic of hemophilia. The poor consumption of prothrombin by the one stage test is also considered to indicate hemophilia in the absence of thrombocytopenia. The relatively normal consumption by the two stage method, lack of definite family history and failure to respond to the administration of fresh plasma, with a good response to protamine, are against such a diagnosis. This condition might be called “hemophilia” provided this designation is used as Tocantins defines it: to indicate the presence of excess antithromboplastin in the blood. This might also explain Allen’s finding of an increased protamine titration test in some of the “hemophiliacs” which he studied.

The source of the heparinoid substance in this patient is completely obscure. He had received neither irradiation nor nitrogen mustard therapy. The existence of an idiopathic hyperplasia of the mast cells throughout his body is a theoretical possibility. The possibility of familial hyperheparinemia remains although, in this study, two brothers, three cousins and an aunt on the maternal side, were found to have normal clotting times.

Conclusions

1. The study of a patient is presented in whom an anticoagulant, having antithromboplastic and antithrombin properties, caused a serious blood coagulation defect.

2. This anticoagulant had many of the properties of heparin and administration of 200 mg. of protamine sulfate intravenously reduced the clotting time from 106 to 43 minutes.

3. The existence of an idiopathic “hyperheparinemia” in this patient is postulated.

References

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