A Familial Hemorrhagic Diathesis due to an Antithrombin

By G. Malcolm Brown, N. E. Diamant, P. R. Galbraith
and W. E. C. Wilson

In a review published in 1955, Alexander stated that he knew of no well-documented case of disturbed hemostasis in which excessive antithrombin activity had been the primary disorder. In 1955, Chevalier et al. reported briefly on three patients in whom purpura was accompanied by an increased capacity of serum to destroy thrombin during incubation at 37°C; in at least one of these patients the hemostatic defects were accompaniments of an acute febrile episode. Loeliger and Hers have described chronic antithrombinemia in a case of rheumatoid arthritis with marked hypergamma-globulinemia. More recently, the occurrence of circulating antithrombins has been described in many disease states. We report now on a familial hemorrhagic diathesis in which increased antithrobin activity was an isolated defect.

Clinical Data

The family studied were Mohawk Indians of mixed blood who had long been considered "bleeders" by their kinsmen. Our substantiation of this lay assessment followed on the discovery of a prolonged clotting time in a child admitted for tonsillectomy. The genetic chart (fig. 1) shows that the abnormality has occurred in males and females and that it has been passed on by both males and females who do not have the stigma either clinically or on laboratory investigation. The clinical picture is usually mild with epistaxis being the most common and often the only manifestation. Excessive bleeding has followed tooth extraction, and one 3 year old boy who bled severely following tonsillectomy died 14 hours postoperatively. Purpura, bleeding into joints, hematemesis, melena, hematuria and excessive menstrual bleeding have not occurred. Physical examination has not revealed any abnormality related to the hemorrhagic disorder.

The tonsillectomy which had a fatal outcome was the only example of surgery in those subjects who were considered to be bleeders either on clinical or laboratory examination. None of the females known by us to have the defect have so far borne children.

Information has been obtained concerning 46 members of the family and laboratory studies have been carried out on 18. In all cases in which the history was considered to be positive and in which there was an opportunity for laboratory investigation, the laboratory studies confirmed the presence of excessive antithrombin activity. In all cases in which excessive antithrombin activity was demonstrated in the laboratory, there was a clinical history of abnormal bleeding.

Case Reports

R. H., a female North American Mohawk Indian (whose paternal great-grandfather was Scottish), aged 6 years, was admitted for tonsillectomy because of recurrent upper respiratory tract infections and enlarged, moderately injected tonsils. A routinely performed clotting time was significantly prolonged and she was discharged. One month later, investigation on the hematologic service established that from the age of 2 the patient had been subject to frequent epistaxes, particularly in the summer. These episodes...
were from 30 to 60 minutes in duration. On one occasion she had bled for 24 hours following tooth extraction. Enlargement of the tonsils was the only significant physical finding. Chest x-ray was negative.

L. H., aged 9 years and a sister of R. H., was admitted for investigation at the same time as her sister as she too had been found to have a prolonged clotting time. Since infancy she had had frequent epistaxes, particularly during the summer when they were said to occur every day. Bleeding was described as small in amount and ceased spontaneously after 1 or 2 hours rest. She had bled for 24 hours following a tooth extraction 3 years before. Physical examination and chest x-ray were negative.

LABORATORY INVESTIGATIONS

Methods

The following methods were used in the investigation of hemostatic function: bleeding time (Duke)\(^6\), tourniquet test (MacFarlane and Biggs\(^7\)), standard clotting time (SCT) (Mayer\(^8\)), plasma fibrinogen (King and Wootton\(^9\)), one-stage prothrombin time (Quick\(^10\)), two-stage prothrombin time (Quick\(^11\)), prothrombin consumption (Stefanini and Dame-shek\(^12\)), thromboplastin generation test (Biggs and Douglas\(^13\)), BaSO\(_4\) adsorption\(^14\), and a test for anti-tissue-thromboplastin (Biggs and MacFarlane\(^15\)).

Two tests were used to determine antithrombin activity. One of these was the method of Biggs and MacFarlane.\(^13\) In the other test, samples of plasma or of serum were added to a thrombin solution and aliquots removed at intervals for addition to BaSO\(_4\)-treated plasma. The plasma used for testing was fresh and the serum was obtained by centrifugation after venous blood had been allowed to clot at room temperature for 1 hour. One-tenth ml. of serum or plasma was added to a tube containing 0.9 ml. thrombin solution in a water-bath at 37 C. The thrombin solution was prepared by diluting Topical Thrombin (Parke Davis Co.) in saline to a concentration which caused clotting in 9 to 10 seconds.
Two female children aged 6 (L. H.) and 9 (R. H.) were studied intensively (Table 1). In 12 other subjects, tests for antithrombin activity were carried out, and in a total of 18 subjects the standard clotting time was determined. A number of experiments have been carried out to characterize the abnormal factor involved.

In the subjects studied intensively, the bleeding time, tourniquet test,
plasma fibrinogen, prothrombin time and thromboplastin generation test were normal. Normal correction of factor V-deficient plasma and of factor VII-deficient plasma was demonstrated. There was no evidence of an anti-tissue-thromboplastin and an intravenous injection of protamine sulphate did not correct the abnormal standard clotting time. Adding toluidine blue to plasma did not diminish its antithrombin activity. The two-stage prothrombin time was abnormal (fig. 2) and prothrombin consumption was normal. The antithrombin test of Biggs and MacFarlane was definitely abnormal (fig. 3). When the antithrombin activity of serum or plasma was determined by the method involving incubation of thrombin for 1, 5, 10, and 15 minutes, a striking difference from normal was found (fig. 4).
Table 2.—Antithrombin Tests in Normal Subjects and in Members of the Affected Family

<table>
<thead>
<tr>
<th>SEX</th>
<th>AGE</th>
<th>HEMORRHAGIC MANIFESTATIONS</th>
<th>Clotting time (secs) of Ba Plasma following addition of 0.4 ml. of serum thrombin mixture after its incubation for 1, 5, 10 &amp; 15 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>8</td>
<td>Epistaxes</td>
<td>14 33 105 575</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>Epistaxes</td>
<td>13 32 85 450</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>Epistaxes</td>
<td>13 33 94 475</td>
</tr>
<tr>
<td>M</td>
<td>82</td>
<td>None</td>
<td>12 19 29 47</td>
</tr>
<tr>
<td>F</td>
<td>72</td>
<td>None</td>
<td>13 25 58 123</td>
</tr>
<tr>
<td>F</td>
<td>54</td>
<td>None</td>
<td>11 24 36 58</td>
</tr>
<tr>
<td>F</td>
<td>30</td>
<td>None</td>
<td>11 20 30 123</td>
</tr>
<tr>
<td>F</td>
<td>31</td>
<td>None</td>
<td>13 23 49 103</td>
</tr>
<tr>
<td>F</td>
<td>7</td>
<td>None</td>
<td>13 28 75 214</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>Epistaxes</td>
<td>3 33 94 475</td>
</tr>
<tr>
<td>M</td>
<td>82</td>
<td>None</td>
<td>12 19 29 47</td>
</tr>
<tr>
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<td>Epistaxes</td>
<td>3 33 94 475</td>
</tr>
<tr>
<td>M</td>
<td>82</td>
<td>None</td>
<td>12 19 29 47</td>
</tr>
</tbody>
</table>

20 Normal males & females age 20 to 60 of both sexes

22 to 30 36 to 75 60 to 204

Table 3.—Comparison of Antithrombin Activity in Normal and in L.H.'s Plasma, Serum, Ba-plasma, Frozen Plasma and Heated Plasma

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Antithrombin Substrate</th>
<th>Thrombin (1000u/cc)</th>
<th>1 min.</th>
<th>5 min.</th>
<th>7 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5cc normal plasma</td>
<td>0.1cc</td>
<td>7.8</td>
<td>10.3</td>
<td>12.9</td>
</tr>
<tr>
<td>2</td>
<td>0.5cc L.H. plasma</td>
<td>0.1cc</td>
<td>10.3</td>
<td>17.5</td>
<td>26.8</td>
</tr>
<tr>
<td>3</td>
<td>0.5cc normal serum</td>
<td>0.05cc</td>
<td>7.8</td>
<td>11.5</td>
<td>17.0</td>
</tr>
<tr>
<td>4</td>
<td>0.5cc L.H. serum</td>
<td>0.05 cc</td>
<td>10.1</td>
<td>16.7</td>
<td>29.4</td>
</tr>
<tr>
<td>5</td>
<td>0.5cc normal Ba-plasma</td>
<td>0.1cc</td>
<td>7.2</td>
<td>8.3</td>
<td>9.2</td>
</tr>
<tr>
<td>6</td>
<td>0.5cc L.H. barium plasma</td>
<td>0.1cc</td>
<td>6.1</td>
<td>7.7</td>
<td>9.8</td>
</tr>
</tbody>
</table>

The results of the tests for abnormal antithrombin activity in the other 12 subjects studied in the laboratory are set out in table 2 along with the clinical findings.

The antithrombin activity of serum was evidence that the abnormal factor was not consumed or destroyed during blood coagulation. Other experiments showed that it was adsorbed on BaSO₄, and that it was stable during storage at −20°C for 10 days but was destroyed by heating to 56°C for 5 minutes (table 3).
A FAMILIAL HEMORRHAGIC DIATHESIS

DISCUSSION

The abnormal antithrombin found in these cases has been incompletely characterized but our preliminary results show that its activity is not similar to the antithrombin activities which Seegers, Johnson and Fell suggested be referred to by the numbers I, II, III, and IV. Using this nomenclature, antithrombin I is fibrinogen: antithrombin activity was present in the serum in our cases. Antithrombin II was the term used to denote the antithrombin activity of heparin and plasma cofactor: the antithrombin factor in our cases was not neutralized in vivo by protamine sulphate or in vitro by protamine sulphate or by toluidine blue. Antithrombin III (which Monkhouse, France and Seegers later thought might be the same substance as plasma cofactor) referred to a substance in plasma which destroyed thrombin independent of heparin, was not adsorbed on a wide variety of agents (Seegers et al.) and was resistant to heating to 60 C. for 3 minutes (Seegers et al.): the antithrombin demonstrated in the present case was completely adsorbed on barium sulphate and was destroyed by heating to 56 C. for 5 minutes. Antithrombin IV referred to a substance which did not destroy added thrombin: our factor did inactivate added thrombin.

The heat lability of antithrombin found in the present cases distinguished it from the factor which Loeliger and Hers described and named antithrombin V and which was stable on heating to 56 C. for 50 minutes, and it also distinguishes it from the antithrombin demonstrated in brain tissue by Nour-Eldin and Wilkinson. The antithrombin demonstrated in a minority of cases of lupus erythematosus by Lee and Sanders was associated with a prolonged one-stage prothrombin time: the difference between this and our cases may or may not be one of degree. Also, the antithrombin demonstrated by Lee and Sanders was labile on storage at 4 C. for 24 hours; as has been noted, our antithrombin was stable during storage at −20 C. for ten days.

The present evidence suggests, therefore, that the abnormal antithrombin activity in our cases is not due simply to the presence of an abnormally large amount of one of the physiologically occurring antithrombins. Neither is it clearly similar to any of the abnormal antithrombins described in some disease states and it is certainly different from the best described of these. Further experiments are required fully to describe its nature and qualities, and the labeling of it would best be deferred until these are complete.

The inheritance pattern is not that of a simple dominant nor a simple recessive. It can be theorized that it is a dominant whose expression is governed by modifier(s), the nature of which are as yet unknown. Clearly the factor is not sex-linked.

SUMMARY

1) A familial hemorrhagic diathesis has been found to be due to an antithrombin.

2) This antithrombin is not fibrinogen, antithrombin II, III, or IV, and is not similar in its characteristics to the antithrombins described in secondary hemorrhagic conditions.
3) The inheritance of this disorder is not sex-linked but is otherwise not clear. The factor may be a dominant, the expression of which is modified by unknown factors.

SUMMARIO IN INTERLINGUA

1. Esseva trovate que un diathese hemorrhagic de occurrentia familial habeva su base in le presentia de un antithrombina.
2. Iste antithrombina non es fibrinogeno; illo non es antithrombina II, III, o IV; e illo non es simile in su characteristicas al antithrombinas describite in conditiones hemorrhagic de supervenientia secundari.
3. Le transmission genetic de iste disordine non es ligate a! sexo. Alteremente illo non es clar. Il es possibile que il se tracta de un factor dominante, le expression del qual es modificate per incognoscite factores.

REFERENCES

A FAMILIAL HEMORRHAGIC DIATHESIS

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