CIRCULATING ANTICOAGULANTS IN HEMOPHILIA AND IN HEMOPHILIA-LIKE DISEASE

By KARL SINGER, M.D., ERNEST MOND, M.D., JULIAN HYMAN, M.D., and ROBERT C. LEVY, M.D.

SINCE the publication of Lozner, Joliffe and Taylor¹ in 1940, a steadily increasing number of cases with hemorrhagic manifestations caused by a circulating anticoagulant has been reported. This clotting anomaly is demonstrated by the prolongation of the clotting time of normal blood which follows the addition of small amounts of the patient's blood or plasma.

Circulating anticoagulants have been found (1) in patients with evident hemophilia,⁷¹² and (2) arising either de novo or in association with various diseases in individuals who exhibit signs and symptoms indistinguishable from hemophilia.¹, ⁵, ⁷⁻¹² In the first group, the circulating anticoagulant has been considered to develop as a result of immunization following blood transfusions⁴ and is probably directed against the so-called "antihemophilic globulin," a plasma factor lacking in these hemophiliacs but supplied in the transfused blood. In the second group, the circulating anticoagulants seem to be of a different nature. They show variations in their physicochemical properties and mode of action, and the pathogenetic mechanisms involved are not understood at present. It is for this latter group that the designation "hemophilia-like disease"¹¹⁴ should be reserved. So far, 9 well documented cases with this disorder have been described.¹, ⁵, ⁷⁻¹²

In this paper, 2 more cases with a circulating anticoagulant are reported. The first patient with hemophilia-like disease is of interest because his anticoagulant was dialyzable and remained demonstrable for a limited period of time only. The second patient, a 4 year old boy, had been assumed to be a hemophiliac for about three years, although the family history was noncontributory. He had never been tested for the presence of a circulating anticoagulant but showed it when first examined in our laboratory. This observation attests to the great difficulties which may be encountered in the differential diagnosis between hemophilia-like disease and the development of a circulating anticoagulant in true hemophilia. It also points clearly to the necessity of testing for circulating anticoagulants in the routine study of any patients exhibiting the features of a severe clotting defect.

REPORT OF CASES

CASE 1. G. R., a 67 year old white male, was first admitted to Michael Reese Hospital in 1945 with a posterior myocardial infarct. No bleeding tendency was noticed at that time nor at an operation for hemorrhoids several years previously. An apical systolic heart murmur had been known to be present for fifteen years. The family and past histories were otherwise noncontributory. In December 1947 he

From the Department of Hematologic Research, Medical Research Institute, and the Department of Medicine, Michael Reese Hospital, Chicago, Ill.

The Department of Hematologic Research is in part supported by the Michael Reese Research Foundation and the Hematology Research Foundation. Acknowledgment is also made for support of these studies to the Hulda B. and Maurice L. Rothschild Foundation for Scientific Research and the Sol Kline Fund.
was readmitted for a second attack of myocardial infarction. Coincidentally, a normochromic normocytic anemia (Hgb. 7.5 Gm., RBC 2.3 M) was discovered. X-ray examination showed a large duodenal ulcer with a deep crater. The patient improved on bed rest, blood transfusions and an ulcer regimen.

In May 1949, painless gross hematuria was observed which subsided within a few days. There were no other symptoms referable to the genito-urinary system. Physical examination was negative except for the previously observed heart murmur. Liver and spleen were not palpable. Urologic study revealed that the hematuria originated in the right kidney. Routine hematologic tests were entirely normal, except for a Lee-White clotting time of more than 60 minutes. The prothrombin time (one-stage method) was within normal limits. A circulating anticoagulant was found to be present. Special studies to determine the nature and mode of action of this anticoagulant are reported below. Other laboratory findings were as follows: Total protein 7.9 Gm. per cent, albumin 4.0 Gm. per cent, globulin 3.9 Gm. per cent. Fractionation of the globulins\(15\): alpha globulin 1.8 Gm. per cent, beta globulin 0.85 Gm. per cent, and gamma globulin 1.25 Gm. per cent. Serum bilirubin 0.7 mg. per cent, cholesterol 255 mg. per cent with 67 per cent esters, alkaline phosphatase 25 units (normal value up to 15 units\(16\)). The thymol turbidity, thymol flocculation and the Hanger test were slightly elevated. The Kahn test was negative. Blood transfusions had no effect on the clotting time.

One week later tarry stools were observed, and an ecchymotic area appeared over the right tibia unrelated to any known trauma. The hemoglobin value had again decreased to 7.3 Gm. with a RBC of 2.4 M. Several transfusions were given, the hemoglobin and red blood count gradually returning to normal. Three weeks later tests for occult blood in the stools were negative. The clotting time was still over 13 hours.

In July 1949 the patient was readmitted because of progressive severe pain in the right gluteal region where black and blue marks had appeared four days previously. Some ecchymotic areas also were seen over the left elbow. Moderate icterus was noticed about this time, but liver and spleen were not palpable. Laboratory tests again showed a normochromic normocytic anemia (Hgb. 9.0 Gm., RBC 2.8 M). The total proteins were 8.1 Gm. per cent, albumin 3.7 Gm. per cent and globulin 4.4 Gm. per cent. The serum bilirubin was now 9.6 mg. per cent, the cholesterol 240 mg. per cent with only 20 per cent esters, the thymol turbidity 4.6 units, and the thymol and cephalin flocculations moderately elevated. The benzidine test of the stools was positive and bilirubin was found in the urine. A few days after admission a huge ecchymotic area, involving the entire right side of the body from shoulder to knee, developed spontaneously. The liver became palpable and tender, and the tip of the spleen could be felt. The finding of regurgitation jaundice with hepato-splenomegaly was interpreted as a homologous serum hepatitis superimposed on the hemophilia-like disease. The patient was put on a high protein, high carbohydrate diet with vitamin supplements, methionine, and absolute bed rest. After ten weeks the jaundice gradually subsided. The clotting time was still 1 hour and 45 minutes.

Three months later, re-examination of the clotting profile showed a normal coagulation time and disappearance of the circulating anticoagulant. Up to now (April 1950), although additional blood transfusions have been given, repeated tests have not revealed the recurrence of any clotting defect.

_Case 2._ J. McC., a 4 year old white boy, was diagnosed as a case of hemophilia when he developed a hemorrhage into the right knee at the age of one year. Since then he had been hospitalized repeatedly because of hemorrhagic manifestations consisting chiefly of bleeding into joints, melena, epistaxis, and ecchymoses. The family history was noncontributory. He had received transfusions on numerous occasions, but their influence on the clotting time had not been studied. When the patient was seen by us, physical examination was negative except for slight enlargement of the liver. There were no joint deformities. Laboratory findings: Urine: trace of albumin with normal sediment. Liver function tests were essentially normal except for thymol and cephalin flocculations. Total proteins 7.7 Gm. per cent, albumin 4.3 Gm. per cent, globulin 3.4 Gm. per cent (alpha globulin 1.0, beta globulin 1.45, gamma globulin 0.9 Gm. per cent). Serum bilirubin 0.3 mg. per cent, cholesterol 185 mg. per cent with 70.8 per cent esters. Thymol turbidity 0.7 units, thymol flocculation 3 plus, and cephalin cholesterol flocculation 1 plus after twenty-four hours. Blood: Hgb. 12.7 Gm. (82 per cent), RBC 3.9 M, WBC 11,500. Differential count: Polys 51 per cent, nonsegmented 1 per cent, eosinophils 4 per cent, lymphocytes 38 per cent, monocytes 6 per cent. The Lee-White clotting time was more than two hours, the prothrombin time was normal. A test for circulating anticoagulant was positive. Further studies of this factor are reported below.
Investigations of the Clotting Defects

Case 1

1. Routine Clotting Studies. The results of all pertinent tests, except the coagulation time, were within normal limits. Representative data are as follows: Fibrinogen 0.46 Gm. per cent, prothrombin time (Quick) 13.7 seconds, platelet count (Dameshek’s method13) 370,000, bleeding time (Duke) 1½ minutes, tourniquet test negative. The Lee-White clotting time was done in three test tubes (8 x 75 mm.) at room temperature. Values up to 2 minutes are considered to be normal. In our patient the clotting time varied from 40 to over 10 minutes while the anticoagulant was demonstrable. Since disappearance of this factor, values of about 14 minutes have been obtained.

2. Demonstration of a Circulating Anticoagulant. Silicone technic was used in collecting and processing blood samples, but clotting times were determined in uncoated Lee-White tubes.

Table 1.—Demonstration of Circulating Anticoagulant

<table>
<thead>
<tr>
<th>Sample</th>
<th>Normal Blood</th>
<th>Normal Saline</th>
<th>Patient’s Plasma</th>
<th>Clotting Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.0 cc.</td>
<td>0.5 cc.</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>B</td>
<td>1.0 cc.</td>
<td>—</td>
<td>0.5 cc.</td>
<td>47</td>
</tr>
<tr>
<td>C</td>
<td>1.0 cc.</td>
<td>0.3 cc.</td>
<td>—</td>
<td>12½ (hemophilic plasma)</td>
</tr>
</tbody>
</table>

To 1.0 cc. samples of normal blood were added portions of the patient’s plasma. As can be seen from table 1, addition of increasing amounts of this patient’s plasma produced progressive lengthening of the clotting times, whereas hemophilic plasma showed no influence on the clotting time of normal plasma.

These results demonstrate convincingly the presence of a circulating anticoagulant. The onset of the bleeding tendency late in life and its transitory character definitely rule out hemophilia and, together with the finding of an anticoagulant, establish the diagnosis of hemophilia-like disease.

3. Physical Properties of the Anticoagulant. An ultrafiltrate was prepared by placing about 10 cc. of the patient’s serum into a Viscose casing bag and suspending it in a centrifuge tube containing about 40 cc. of normal saline. The tube was centrifuged at low speed for several hours at room temperature. Anticoagulant activity was retained in the ultrafiltrate as indicated by its ability to increase the clotting time of normal blood (table 2). This, to our knowledge, is the first time that a circulating anticoagulant was found to be dialyzable. Further studies revealed that this factor was relatively heat resistant. After the patient’s plasma was kept at 55 C.
for five minutes, the clotting time of normal blood could still be prolonged by it
from 9 to 40 minutes.

4. Test for Antithrombin. Thrombin (Parke-Davis) was added in increasing
dilutions to oxalated plasma (1 part of 0.1 molar sodium oxalate solution to 9 parts of
blood), obtained from the patient and a normal control. The thrombin was dis-
solved in normal saline in correct proportions to give the desired number of units
per 0.1 cc. This solution was mixed with an equal amount of 0.025 molar calcium
chloride, and 0.2 cc. of this mixture was then added with a blow-out pipet to 0.1
cc. of oxalated plasma. A stopwatch was started simultaneously, and the first
appearance of fibrin was determined just as in performing the prothrombin time
test. The results are summarized in table 3.

Within the range of thrombin concentrations tested (1–5 units) no significant
difference was found between the two plasmas, indicating the absence of a potent
antithrombin. Unfortunately this experiment was not carried further, using more
dilute thrombin solutions (below 1 unit), with which a weaker antithrombin still
might have been detected. The question whether the anticoagulant acted as an
antithrombin is, therefore, not settled conclusively, although this possibility
seems unlikely in the presence of a normal prothrombin time.

5. Protamine Titration. Addition of protamine failed to cause clotting of the
patient's blood with concentrations from 10 to 400 micrograms per cc. of blood.
These results indicate that the circulating anticoagulant differed from heparin and
the heparin-like factors recently studied by Allen et al. 19, 20

6. Differential Centrifugation Test. Quick 21 reported that after high centrifugation
the clotting time of recalcified hemophilic plasma is considerably slower than that
obtained by low centrifugation. Similar results were seen with our patient's
plasma (table 4). This test does not seem to help in the differentiation between
hemophilia and hemophilia-like disease as has been noted also by other work-
ers. 9, 12
7. Prothrombin Consumption Test. The one-stage procedure, as described by Quick, was employed. This test is designed to ascertain how much prothrombin has been consumed one hour after coagulation is completed. In our control studies, we have found that, normally, more than 60 per cent of the prothrombin is converted, corresponding to prothrombin times of more than 17 seconds. The value obtained with the patient's serum was only 13 seconds, demonstrating a defective consumption of prothrombin. According to Quick, such a result is to be interpreted as a lack of available thromboplastin.

How thromboplastin is actually formed during the clotting process is still not fully elucidated. Brinkhous believes that thromboplastin is released from the platelets through the action of a plasma factor (thrombocytolysin) which may be identical with the "antihemophilic globulin." Quick postulates that platelets do not contain thromboplastin but an enzyme, which activates a plasma thromboplastin precursor (thromboplastinogen, probably identical with antihemophilic globulin). Several other hypotheses, speculating on an interaction between plasma and platelets, have also been advanced. Since the observed anticoagulant produced a defective prothrombin consumption, it was assumed that it interfered either with the formation or with the utilization of thromboplastin. Therefore, it was decided to investigate these possibilities further.

8. Effect of Antihemophilic Globulin. It has been shown that the plasma factor (antihemophilic globulin) is deficient in hemophilia, and that administration of antihemophilic globulin (Fraction I of Cohn) restores the clotting time to normal in this disorder. We have pointed out above that our patient was not a hemophiliac. Nevertheless, it seemed conceivable that the circulating anticoagulant might have been directed against antihemophilic globulin, and that an excess of the latter could then have overcome the anticoagulant activity. Although such an assumption did not appear likely in view of the ineffectiveness of numerous blood transfusions, antihemophilic globulin* was tried in vivo and in vitro. This particular batch of antihemophilic globulin, when tested in a patient with classic hemophilia, reduced the clotting time from 165 to 35 minutes within three hours. There were no appreciable effects on the clotting times in our patient (tables 5 and 6).

9. Effect of Intact and of Ground-up Platelets. In order to determine whether the anticoagulant acted as an antagonist to the platelet factor involved in thromboplastin formation, the effect of intact and of ground-up platelets was investigated.

(a) A suspension of intact platelets was prepared by centrifuging 20 cc. of ox...
lated blood at low speed for five minutes. The platelet-rich plasma was respun at 3000 RPM for thirty minutes in siliconized tubes. The supernatant plasma was then discarded, the pellet of platelets washed several times with normal saline, re-centrifuged, and again suspended in 1 cc. of saline. Addition of 0.5 cc. of this suspension to 1.0 cc. of the patient's whole blood caused a moderate, but definite, reduction of the clotting time (table 7). (b) Similar experiments were performed with an aqueous platelet extract* prepared as described by Mann et al. This involved obtaining platelets as above and, after repeated washings, thoroughly triturating the pellet in a mortar. The homogenate was then dissolved in a solution of normal saline containing 0.001 molar sodium oxalate. The final volume was adjusted so that 1.0 cc. corresponded to platelet extract derived from 15 cc. of plasma. Addition of such a platelet extract to the patient's blood returned the clotting time to normal. An extract prepared from the patient's own platelets was equally effective (table 8).

Table 9 shows that the same platelet extract also neutralized the anticoagulant activity of the ultrafiltrate prepared from the patient's plasma.

As can be clearly seen from these results addition of intact, and even more markedly so of ground-up, platelets overcame the activity of the anticoagulant. Quick and Stefanini have suggested that in hemophilia-like disease the anticoagulant activity of the ultrafiltrate prepared from the patient's plasma.

* For the sake of convenience the terms "platelet extract" and "homogenate" will be used interchangeably.

---

### Table 5.—Effect of Antihemophilic Globulin in Vivo

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Clotting Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before injection</td>
<td>157</td>
</tr>
<tr>
<td>1 hr. after injection</td>
<td>145</td>
</tr>
<tr>
<td>4 hrs. after injection</td>
<td>over 120*</td>
</tr>
</tbody>
</table>

* Only first tube clotted.

### Table 6.—Effect of Antihemophilic Globulin in Vitro

<table>
<thead>
<tr>
<th>Patient's Blood</th>
<th>Normal Saline</th>
<th>Antihemophilic Globulin</th>
<th>Clotting Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.5</td>
<td>—</td>
<td>150</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>0.5</td>
<td>150</td>
</tr>
</tbody>
</table>

### Table 7.—Effect of Platelet Suspension

<table>
<thead>
<tr>
<th>Patient's Blood</th>
<th>Normal Saline</th>
<th>Platelet Suspension</th>
<th>Clotting Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.5</td>
<td>—</td>
<td>150</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>0.5</td>
<td>50</td>
</tr>
</tbody>
</table>
may inhibit the action of the platelet enzyme on the plasma factor (thromboplastinogen). This interpretation represents a definite possibility and, at first glance, seems to be borne out by our experiments. Control studies with hemophilic blood, however, demonstrate that evaluation of the effect of platelet extract is much more difficult. As can be seen from table 10, platelet homogenates are also capable of restoring the clotting time of hemophilic bloods to normal values. In order to explain this latter finding, it must be realized that platelet extract is certainly a complicated reagent. Ware et al. found it to contain traces of thromboplastin, an accelerator factor similar in action to serum Ac-globulin, and a substance which apparently enhances the action of thrombin on fibrinogen. Whether other factors are also present in the extract is not as yet known. It is well established that thromboplastin can clot hemophilic blood; even very small amounts of tissue juice contamination in venipuncture cause a considerable reduction of the coagulation time. Since the platelet extract used was highly concentrated, the question arose whether its clot-promoting ability could be attributed to its thromboplastin content. When

<table>
<thead>
<tr>
<th>Patient's Blood</th>
<th>Normal Saline</th>
<th>Platelet Extract</th>
<th>Clotting Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>1.0</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>13</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>0.5</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 8.—Effect of Platelet Extract

<table>
<thead>
<tr>
<th>Normal Blood</th>
<th>Normal Saline</th>
<th>Platelet Extract</th>
<th>Ultra Filtrate</th>
<th>Clotting Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>—</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>140</td>
</tr>
<tr>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 9.—Effect of Platelet Extract on Ultra Filtrate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hemophilic Blood</th>
<th>Normal Saline</th>
<th>Platelet Extract</th>
<th>Clotting Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
<td>35</td>
</tr>
<tr>
<td>1.0</td>
<td>0.3</td>
<td>—</td>
<td>0.3</td>
<td>9</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>0.3</td>
<td>—</td>
<td>44</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>0.3</td>
<td>14</td>
</tr>
<tr>
<td>B</td>
<td>1.0</td>
<td>0.3</td>
<td>—</td>
<td>150</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>0.3</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>C</td>
<td>1.0</td>
<td>0.3</td>
<td>—</td>
<td>150</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>0.3</td>
<td>—</td>
<td>8</td>
</tr>
</tbody>
</table>
the thromboplastic activity of our platelet extracts was measured on normal plasmas with the one-stage prothrombin time procedure, it corresponded to a dilution of thromboplastin (Maltine) of about 1:800. As table II demonstrates, such a thromboplastin concentration was well above the minimal amount necessary to restore the clotting time of hemophilic blood to normal. It, therefore, appears quite likely that platelet extract acted by virtue of its thromboplastic activity.

10. Mode of Action of the Anticoagulant. It may be recalled that addition of platelet extract produced a normal clotting time of the patient’s blood. The problem now remains to apply the information about the thromboplastic activity of the platelet extract to an understanding of the mode and site of action of the anticoagulant. Our studies seem to leave a choice of two possibilities. (1) The anticoagulant may have been a direct antithromboplastin, or (2) it may have interfered with the formation of thromboplastin from platelet and plasma factors. In either case platelet homogenate can be considered to have exerted its effect by providing sufficient thromboplastic activity for the clotting process.

Table II.—Effect of Thromboplastin Dilutions on Hemophilic Blood

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hemophilic Blood</th>
<th>Normal Saline</th>
<th>Thromboplastin Dilution (0.5 cc.)</th>
<th>Clotting Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.0</td>
<td>0.3</td>
<td>—</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>—</td>
<td>1:500</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>—</td>
<td>1:800</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>—</td>
<td>1:1000</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>—</td>
<td>1:1000</td>
<td>14</td>
</tr>
<tr>
<td>B</td>
<td>1.0</td>
<td>0.3</td>
<td>—</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>—</td>
<td>1:500</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>—</td>
<td>1:800</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>—</td>
<td>1:1000</td>
<td>21</td>
</tr>
</tbody>
</table>

If the assumption is made that the extract worked solely through its thromboplastic activity, the anticoagulant could not have been anything but a relatively weak antithromboplastin. It is conceivable, however, that the anticoagulant may have been present in just sufficient concentration to neutralize the patient’s own thromboplastin. In this case platelet extract simply furnished the necessary thromboplastic agent. It was planned to rule out this mechanism by titrating the patient’s plasma with increasing dilutions of tissue thromboplastin. Unfortunately the anticoagulant had disappeared by that time.

Considering the other possibility, namely that thromboplastin formation was inhibited, the platelet extract most probably acted in a similar fashion, i.e., by supplying the thromboplastin whose production the anticoagulant prevented. We are inclined to regard this mode of action of the anticoagulant as the more likely one.

Case 2

1. Routine Clotting Studies. Again, all pertinent tests except the clotting time were within normal limits: Prothrombin time 10.9 seconds, platelets 350,000, bleeding time 2½ minutes. The coagulation time never was less than 90 minutes.
2. **Demonstration of a Circulating Anticoagulant.** The method used differed from that in the first case and may be recommended as a standard technic because of its relative simplicity. Blood is drawn simultaneously by clean venipuncture with No. 20 needles from the patient and a normal control. A stop-watch is started at the first appearance of blood in the syringes; slight differences in time in obtaining the blood are insignificant in the presence of a potent anticoagulant. Three-tenths cc. of the patient's blood are put into each of three Lee-White clotting tubes previously marked at the 0.3 and 1.3 cc. levels. One cc. of the normal blood is then added, each tube is inverted once and thereafter the clotting time determined in the usual manner. The clotting time of the control subject is measured at the same time in an identical fashion, but 0.3 cc. of normal saline are added to 1 cc. of blood in each tube. The theoretic possibility exists that group incompatibility between the two tested bloods, with clumping and/or lysis of red cells, may interfere with the test. We have not met with such difficulties, but it may be advisable to use a homologous control subject if doubtful results are encountered. Table 12 shows the marked anticoagulant effect exerted by the patient's blood.

| Table 12.—Demonstration of Circulating Anticoagulant |
|----------------|----------------|----------------|----------------|
| Sample        | Normal Blood   | Patient's Blood| Normal Saline  |
|               | cc.            | cc.            | cc.            |
| A             | 1.0            | —              | 0.3            | 14 |
|               | 1.0            | 0.3            | —              | 110 |
| B             | 1.0            | —              | 0.3            | 19 |
|               | 1.0            | 0.3            | —              | 60 |

3. **Physical Properties of the Anticoagulant.** In contradistinction to our first case, the anticoagulant of the second patient was not dialyzable. The factor was not inactivated by exposure to temperature of 55 C. for ten minutes, and to 37 C. for sixteen hours.

4. **Prothrombin Consumption Test.** Determination of the prothrombin consumption time was unsuccessful on two occasions because the patient's plasma, collected in siliconized syringes, failed to clot in glass tubes even after sixteen hours. Addition of 0.1 cc. of platelet extract to 0.5 cc. of such plasma, however, caused clotting within sixty minutes. The prothrombin consumption time determined on this sample after removal of the clot was 12.3 seconds and 12.2 seconds one hour later. As in the first case, this abnormal result seems to signify a disturbance in either thromboplastin formation or utilization.

5. **Test for Antithromboplastin.** If the anticoagulant substance acted as direct antithromboplastin, one would expect to find a marked difference between the clotting times of the patient's and normal recalciﬁed plasmas with the use of increasing dilutions of thromboplastin. The technic employed was identical in all respects with that of the routine prothrombin time procedure except for substituting saline dilutions of thromboplastin for the undiluted reagent (thromboplastin Maltine).

The results of these tests, including three normal plasmas as controls, are tabulated in table 13. Since differences between the normal plasmas were found with
higher thromboplastin dilutions, evaluation becomes hazardous. Nevertheless, the marked prolongation of the coagulation of the patient’s plasma with very high dilutions of thromboplastin could be interpreted to be the effect of a weak antithromboplastin. However, these findings do not rule out a disturbance of the patient’s own thromboplastin formation, lack of which may also increase the clotting time if insufficient extraneous thromboplastic material is supplied.

We are aware of the shortcomings of using heterologous thromboplastin, especially in view of the observations of Fantl and Nance, who demonstrated antithromboplastic activity with human, but not with rabbit, thromboplastin in a similar case. We were unable to repeat this experiment with thromboplastin from human sources.

6. Precipitin Test. Serum precipitins against antihemophilic globulin and normal plasmas have been demonstrated in the past in hemophiliacs developing a circulating anticoagulant. Since antihemophilic globulin was not available, normal lyophilized plasma—in concentrations from 3:1 to 1:2056—was used as antigen with the routine and a modified gelatin technic. No precipitins were detectable.

Table 13.—Test for Antithromboplastin

<table>
<thead>
<tr>
<th>Dilution of Thromboplastin</th>
<th>Clotting Time in seconds</th>
<th>Patient</th>
<th>Control A</th>
<th>Control B</th>
<th>Control C</th>
</tr>
</thead>
<tbody>
<tr>
<td>undiluted</td>
<td></td>
<td>11 1/2</td>
<td>12 1/2</td>
<td>12 1/2</td>
<td>12 1/2</td>
</tr>
<tr>
<td>1:200</td>
<td></td>
<td>42</td>
<td>39</td>
<td>44</td>
<td>—</td>
</tr>
<tr>
<td>1:500</td>
<td></td>
<td>56</td>
<td>48</td>
<td>61 1/2</td>
<td>—</td>
</tr>
<tr>
<td>1:800</td>
<td></td>
<td>72</td>
<td>57</td>
<td>73</td>
<td>72 1/2</td>
</tr>
<tr>
<td>1:1000</td>
<td></td>
<td>80</td>
<td>61</td>
<td>79</td>
<td>76</td>
</tr>
<tr>
<td>1:2000</td>
<td></td>
<td>103 1/2</td>
<td>77</td>
<td>97 1/2</td>
<td>97</td>
</tr>
<tr>
<td>1:4000</td>
<td></td>
<td>163</td>
<td>107</td>
<td>118</td>
<td>104</td>
</tr>
<tr>
<td>1:8000</td>
<td></td>
<td>219</td>
<td>119</td>
<td>—</td>
<td>117</td>
</tr>
</tbody>
</table>

Only a positive test would have been contributory here; negative results must be considered inconclusive, since the antigen was neither purified nor concentrated. Our efforts to prove the diagnosis of a pre-existing hemophilia were, therefore, not successful.

In summary, the anticoagulant in this patient—as in our first case—probably interfered with thromboplastin formation, but slight antithromboplastic activity could not be excluded.

Discussion

Although the literature contains reports of patients with a hemorrhagic diathesis and a prolonged clotting time who apparently did not have hemophilia (hemophilia in females, “acquired” hemophilia beginning late in life in males), it is only recently that an understanding of some of these clinical pictures has become possible by the demonstration of circulating anticoagulants. While such cases are not seen frequently, they are by no means medical curiosities. Tzanck, Soulier and
Blatrix found 3 patients with circulating anticoagulants among 370 hemorrhagic syndromes. Conley et al. discovered 8 instances among 41 subjects studied. Our own cases were found by testing a group of 9 individuals showing hemorrhagic diatheses with a prolonged clotting time. By considering only the sufficiently well investigated patients with potent anticoagulants, we have been able to collect 18 authentic cases, including our own, 12 of which have been summarized recently by Dreskin and Rosenthal. Of these, 7 were proven hemophiliacs; in 10, hemophilia could be ruled out on the basis of sex or clinical history, whereas in one (our Case 2) the distinction between hemophilia and hemophilia-like disease could not be made. Clinically our two observations differ from those previously reported in that the first anticoagulant was transitory in nature, while the second occurred in a 4-year-old child. The syndrome has not been encountered before in this age group.

The most frequent single disease entity present in the 18 patients with circulating anticoagulants was hemophilia, which was found in 40% of the group. Craddock and Lawrence attribute the pathogenesis of anticoagulants in hemophilia to isoimmunization. Considering hemophilia as being caused by a lack of antihemophilic globulin, blood transfusions, supplying antihemophilic globulin, may stimulate production of antibodies directed against this factor. According to general immunologic principles such isoimmunization could occur only if antihemophilic globulin was completely absent in the recipient and, therefore, would account for the relative rarity of this complication in hemophiliacs. Precipitin tests against purified antihemophilic globulin have been used to demonstrate such antibodies. Positive results were obtained in 2 cases, while in 2 others such immune bodies were not demonstrable.

It is possible that our second patient originally had hemophilia. The onset of clinical manifestations in this particular age group does not help in the differential diagnosis between hemophilia with an anticoagulant and hemophilia-like disease due to an anticoagulant; if the bleeding tendency had made its first appearance much later in life, hemophilia could have been excluded. The absence of a positive family history is also not contributory, since inheritance can not be established in about one-third of all cases with hemophilia. Unfortunately, the effect of blood transfusions on the clotting time had never been studied previously, and no test for the presence of an anticoagulant had been performed when the clotting anomaly was first discovered. The precipitin test in our patient was inconclusive; if positive it could have clarified the problem whether the anticoagulant arose de novo or was related to a pre-existing hemophilia.

* Since this paper has been accepted, Frommeyer et al. (Blood: 401, 1950) have reported the development of anticoagulants most likely directed against the antihemophilic globulin in 5 cases of classic hemophilia. In 2 of these patients, who received no therapy over extended periods of time, the anticoagulant effect decreased in one and disappeared in the other. These investigators also found that transfusions of very large amounts of fresh blood were capable of overcoming the anticoagulant activity and increasing the rate of clotting in these hemophiliacs.
The differentiation between hemophilia, hemophilia complicated by an anticoagulant, and hemophilia-like disease caused by one is of more than academic interest. While specific therapy is available for the clotting defect in hemophilia in the form of normal plasma or antihemophilic globulin, the presence of an anticoagulant renders such treatment ineffective.* Since hemophilia-like disease is apparently not a hereditary disorder, a well supported diagnosis permits the prediction that propagation of the disease is unlikely—an important consideration when dealing with the family of the patient. Finally, as can be seen from our first case, circulating anticoagulants in hemophilia-like disease may disappear spontaneously with complete restoration of the normal clotting mechanism. The prognosis, therefore, may be quite different from that of hemophilia and hemophilia with an anticoagulant, both incurable conditions. In view of these implications it is important to place any patient presenting the clinical picture of hemophilia into one of these three categories. This can be achieved only by testing routinely for a circulating anticoagulant at the earliest possible moment in every one of these cases.

Whereas the immunologic hypothesis offers an attractive explanation of the development of a circulating anticoagulant in hemophilia, the pathogenesis of the anticoagulants responsible for hemophilia-like disease remains obscure. Attempts have been made to apply a similar etiologic mechanism to these cases and pregnancy has been implicated to play a role in a manner analogous to isoimmunization with the Rh factors. In the 4 females studied, the time intervals between parturition and onset of the bleeding manifestations have been three weeks, 9 and three, 12 four 8 and twelve months, 13 respectively. These data are not too convincing, and no such etiology can be invoked to explain satisfactorily the occurrence in the 6 male patients. In 5 of these cases the anticoagulant was associated with the following disorders: generalized lymph node tuberculosis and anthracosis 1; pemphigus 11; chronic nephritis 6; dermatitis herpetiformis (Duhring-Brocq) 7; coronary artery disease and peptic ulcer. A positive serology was found in 2 of these cases. Hodgkin's disease and multiple myeloma may possibly be added to this list; a similar syndrome has been observed in these entities, 26 although no tests for circulating anticoagulants were actually carried out. It is difficult to detect a common denominator for these varied disorders, and much more information is needed for proper evaluation of a possible causal relationship.

Electrophoretic studies in hemophilia-like disease showed abnormalities in the albumin fraction in one instance 13 and of the gamma globulins in another 7; in hemophilia with an anticoagulant the three examined plasmas revealed pathologic changes in the gamma globulins. 5-4 The anticoagulant in our first patient is the only one so far observed to be both dialyzable and transitory in nature. Rosenthal et al. 35 described an "acquired" hemophilia in a female in whom the hemorrhagic tendency and the prolonged clotting time also disappeared after thirteen years. In retrospect, this clinical picture has been interpreted as probably being caused by a transient anticoagulant. 12 One may speculate whether a connection exists between the physical property of the factor and its spontaneous disappearance. These ob-

* See, however, preceding footnote.
servations also point against an immunologic origin of at least some of the anti-
coagulants in hemophilia-like disease and support the proposed classification of
this malady as a separate entity.

Most of the anticoagulants investigated apparently interfere with either forma-
tion or utilization of thromboplastin. In uncomplicated hemophilia, as it is now
widely accepted, there also is a deficiency of available thromboplastin.36, 37 It is
understandable, therefore, that these disorders show essentially the same labora-
tory features, i.e., increased clotting time, normal prothrombin time, and a patho-
logic prothrombin consumption test. Finding an increased prothrombin time in
2 of these cases with hemophilia-like disease5, 9 indicates an even more complex
action of some of these anticoagulants, which may also inhibit the transformation
of prothrombin to thrombin. Such an involved mechanism must be postulated,
since assumption of isolated antiprothrombic activity cannot account for the
markedly increased coagulation time in the presence of an only moderately pro-
longed prothrombin time. It still remains conceivable that anticoagulants may
act against other factors of the clotting system. Conley and co-workers,33 for
instance, have mentioned 2 patients with severe hepatitis in whom antithrombic
activity was demonstrated. None of these anticoagulants were heparin or heparin-
oid substances,19 as protamin titrations have been consistently negative.

We are obviously only at the beginning of an understanding in the field of
circulating anticoagulants. If the simple test as used in our second case is applied
routinely to all patients with a definitely increased clotting time, many more
cases will undoubtedly be discovered, and more material for clinical, physiologic
and chemical studies will become available. One may even speculate whether a
new type of therapeutic anticoagulant may not be found through such inves-
tigations.

Summary

1. Hemophilia-like disease may be defined as a hemorrhagic diathesis charac-
terized by a prolonged clotting time and the presence of a circulating anticoagulant.
Clinical data, such as occurrence in females or onset late in life in males, are of
great value in establishing the diagnosis. It is to be distinguished from hemophilia
complicated by the development of an anticoagulant following blood transfusions.
Differentiation of these disorders is of practical significance because hemophilia-
like disease is not hereditary and the clotting anomaly may disappear sponta-
neously.

2. Two cases with circulating anticoagulants are reported. In the first patient
with hemophilia-like disease the anticoagulant was dialyzable and present for a
limited period of time only. In the second patient, a 4 year old boy, the distinction
between hemophilia-like disease due to an anticoagulant and pre-existing hemo-
philia with a complicating anticoagulant could not be made.

3. Analysis of the mode of action of the anticoagulants in these 2 cases showed
interference with the formation of thromboplastin, although some antithrombo-
plastic activity could not be excluded.

4. A simple technic for testing for circulating anticoagulants is described. It is
CIRCULATING ANTICOAGULANTS IN HEMOPHILIA

recommended that this test be performed routinely in all patients with a bleeding tendency and a prolonged clotting time.

ADDENDUM

Case 2. J. McC. has since been treated with cortisone, 100 mg. daily for a period of eighteen days in an attempt to inhibit the production of a possible immunologic anticoagulant. There has been no change in the clotting time or in the ability of the patient's blood to prolong the clotting time of normal blood.

ACKNOWLEDGMENTS

We are indebted to Dr. Raphael Isaacs for permission to study his private patient. We gratefully acknowledge the assistance of Miss Helen MacLean from the Department of Bacteriology in performing the precipitin tests.

REFERENCES

SINGER, MOND, HYMAN AND LEVY

AMERICAN JOURNAL OF HEMATOLOGY

volume 4, number 4

MARCH 1970

Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Correspondence.


Correspondence.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.


Proteinuria in patients with acute leukemia.

CIRCULATING ANTICOAGULANTS IN HEMOPHILIA AND IN HEMOPHILIA-LIKE DISEASE

KARL SINGER, ERNEST MOND, JULIAN HYMAN and ROBERT C. LEVY