A Plasma Coagulation Defect in Systemic Lupus Erythematosus Arising from Hypoprothrombinemia Combined with Antiprothrombinase Activity

By Samuel I. Rapaport, Sara Beth Ames and Barbara J. Duvall

Conley and Hartmann first called attention to a plasma coagulation disturbance peculiar to patients with systemic lupus erythematosus or closely related dysproteinemias. Its characteristics are a long whole blood and/or recalcified plasma clotting time, a long Quick "prothrombin" time, and the presence of a circulating anticoagulant interfering with prothrombin conversion by either formed blood "thromboplastic" activity or tissue thromboplastin. The anticoagulant has no antithrombic activity and is not neutralized by protamine; thus, it is quite unlike heparin.

The prolonged Quick "prothrombin" time was originally attributed to the anticoagulant's ability to inhibit tissue thromboplastin. However, evidence that these patients may also develop true hypoprothrombinemia has gradually accumulated as they have been studied with more specific prothrombin techniques.

The child with systemic lupus erythematosus to be described in this paper had serious bleeding associated with a severe plasma coagulation disturbance. A profound hypoprothrombinemia was demonstrated by every technic used to measure prothrombin. The hypoprothrombinemia overshadowed evidence of the simultaneous presence of an anticoagulant which interfered with prothrombin conversion by either tissue or blood prothrombinase. A comparison of these findings with those in the literature leads us to conclude that the plasma coagulation disturbances of systemic lupus erythematosus usually stem from a mixture of anticoagulant activity and true hypoprothrombinemia.

REPORT OF CASE

J. G., LACGH No. 980-404, was an 11 year old girl. While hospitalized for lobar pneumonia at the age of three years, she was discovered to have doubtful serology. She was seen at the age of four years because of nosebleeds and fever and again at the age of five years because of fever, headache, stiff neck and lethargy attributed to nonparalytic poliomyelitis.

In July 1956, when she was 10 years old, she was brought to the hospital because of four days of epistaxis and a six months' history of headache, anorexia, recurrent fever and painful ankles and knees. A sedimentation rate of 41 mm. per hour was found and an antistreptolysin titer of 200 units.
She did not return for follow-up studies but reappeared in July 1957 with the complaint again of epistaxis and of swelling and pain in her ankles. Outpatient diagnostic studies were interrupted in early August 1957 by the onset of uncontrollable epistaxis requiring hospitalization.

She gave no other history of unusual bleeding. Her tonsils had been removed elsewhere in November 1956 without abnormal bleeding. She did not bruise easily and had not noticed petechiae. There was no family history of abnormal bleeding.

On physical examination, she appeared chronically ill. Her abnormal physical findings were a temperature of 100.8 F., a pulse of 106 per min., bleeding from the nose and gums and slight cervical and axillary lymphadenopathy.

On laboratory examination, she was found to have a Hgb. of 7.6 Gm. per cent, WBC count of 4,200 per cu.mm. with a normal differential on smear and a platelet count of 184,000 per cu.mm. Blood chemical analyses were: albumin 3.6 Gm. per cent, globulin 2.8 Gm. per cent, cephalin flocculation 3+ in 24 hours, thymol turbidity 3 units, alkaline phosphatase 2.2 Bodansky units, BSP less than 10 per cent in 45 minutes. Her anti-streptolysin titer was 100 units. An electrocardiogram, chest film and bone marrow examination were within normal limits.

Her initial coagulation studies revealed the following: Lee-White clotting time 45 min. (normal less than 15 min.); partial thromboplastin time 160 to 180 sec. (normal 50 to 90 sec.); Quick "prothrombin" time 21 per cent; prothrombin-proconvertin test6 9 per cent; venom-"cephalin" prothrombin assay7 8 per cent; proaccelerin assay 76 per cent; thrombin time 35 sec. (normal 26 sec.); fibrinogen greater than 0.3 Gm. per cent. An antihemophilic globulin (AHC) assay revealed 110 to 140 per cent AHG (normal 70 to 140 per cent). Her chloride serum behaved like normal serum in its ability to correct the abnormal "thromboplastin" generation test of an incubation mixture containing plasma thromboplastin component (PTC)-deficient serum. Her prothrombin-proconvertin time failed to improve after 50 mg. of vitamin K₁ oxide given intravenously. Her tourniquet test was negative; her bleeding time (Duke method) was 6⅔ min., and her platelet count (Rees-Ecker method) was 184,000 per cu.mm.

Profuse bleeding from her nose and oozing from her gums persisted despite the use of packs, cauterization, intramuscular and intravenous vitamin K preparations and repeated

*There is no standard nomenclature for the clotting activities. The following terms are used in this paper. Tissue thromboplastin is the activity found in aqueous extracts of brain tissue. Proconvertin is a stable prothrombin conversion substance present in normal plasma and serum, necessary for clotting with tissue thromboplastin, but not for clotting with Russell’s viper venom or blood thromboplastin. Stuart factor is a second stable prothrombin conversion substance resembling proconvertin closely but different in that it is necessary for clotting in the presence of all three of the above-cited thromboplastic activities. Convertin is the name of the activity formed when tissue thromboplastin and proconvertin (and probably Stuart factor) react together in the presence of calcium ions. Proaccelerin is the name used for the labile accelerator of thrombin formation present in plasma after it is treated with the prothrombin adsorbents but not present in aged human serum; synonyms are plasma Ac-globulin and labile factor. Accelerin is the name used for the increased accelerator activity produced by adding a small amount of thrombin to adsorbed plasma; synonym is serum Ac-globulin. Tissue prothrombinase refers to the final prothrombin converting activity that results from the simultaneous presence of or interaction of convertin and accelerin.

The term blood thromboplastin is reserved for a hypothetical activity which may arise in shed blood and which is equivalent to the clotting activity of aqueous tissue extracts. Blood prothrombinase is the name used for the final prothrombin converting activity generated in blood free of tissue products. Thus, blood prothrombinase is the converting activity generated in the incubation mixture of the so-called "thromboplastin" generation test.

When, in referring to the work of others, these terms are used differently they are given in quotation marks.
transfusions of bank and fresh blood. During her second week of hospitalization, she
developed chest pain, x-ray evidence consistent with pneumonia, a purpuric rash on her
extremities, and left flank pain followed by gross hematuria. She ran an intermittent fever.

At first we were at a loss to explain her bleeding and profoundly disturbed "pro-
thrombin" times. She did not have liver disease and was not vitamin K-deficient. Congenital
hypoprothrombinemia was considered. Her history of a tonsillectomy without bleeding
was against this, and the diagnosis was discarded when her anticoagulant was discovered.
Systemic lupus erythematosus was thought of when she developed findings of systemic
disease and her serology was reported as positive. Several positive L.E. cell tests established
the diagnosis.

She was started on oral prednisone, 80 mg. daily. She grew worse with persistence of
the above-cited findings and the onset of a left pleural effusion. Brisk epistaxis continued
and her coagulation tests did not improve. Therefore, on the fifth day, massive intravenous
hydrocortisone therapy, 600 mg. daily, was added. The effect was remarkable. Within
24 hours she was afebrile, her chest and flank pain were gone and her gums and nose
stopped bleeding. As table 1 shows, her coagulation tests returned to normal over the
next few days.

Despite this, she lived only a few months longer. She required enormous amounts of
adrenal steroids to suppress lupus activity. As a result, she developed many serious compi-
cations of hyperadrenalcortisonism. Since her coagulation findings remained normal, this
phase of her illness will not be described. Her terminal episode, in March 1958, was one
of chest pain with pulmonary infiltration, headache and irritability.

Systemic lupus erythematosus was found at autopsy along with an unsuspected cryp-
tococcal pneumonitis and meningitis.

**Coagulation Studies**

The human plasma used in these studies was prepared by collecting nine volumes of
venous blood in one volume of 0.1 M sodium citrate solution. Prothrombin was removed
by aluminum hydroxide (Cutter Laboratories) adsorption. All serum was decalcified by
adding one part of 0.1 M sodium citrate solution to five parts of serum. The preparation
of human brain extract thromboplastin, "cephalin," and adsorbed ox plasma has been
described elsewhere. Proaccelerin-deficient substrate plasma was made by Stormorken's
technic. Proconvertin-deficient substrate plasma was kindly furnished by Dr. Paul Aggeler
from his patient, B. C. Thrombin solution (Parke-Davis Bovine Thrombin) was prepared
as described earlier. Accelerin reagent was made by adding dilute thrombin to either
adsorbed ox or human plasma. Proconvertin reagent (which also presumably contained
Stuart factor) was made from normal serum using a technic originally described for
making PTC reagent.

Our technics for the Quick "prothrombin" test, the prothrombin-proconvertin test, the
venom-"cephalin" prothrombin test, the partial thromboplastin time and the plasma

<table>
<thead>
<tr>
<th>Date</th>
<th>Steroid therapy (mg. daily)</th>
<th>Lee-White clotting time (min.)</th>
<th>Proth-preconvert. time (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 14</td>
<td>None</td>
<td>45</td>
<td>8</td>
</tr>
<tr>
<td>Aug. 31</td>
<td>Prednisone started (80 mg.)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Sept. 2</td>
<td>--</td>
<td>45</td>
<td>less than 5</td>
</tr>
<tr>
<td>Sept. 4</td>
<td>Hydrocort. added (600 mg.)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Sept. 7</td>
<td>Hydrocort. reduced (400 mg.)</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>Sept. 10</td>
<td>--</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Sept. 16*</td>
<td>Hydrocort. reduced (150 mg.)</td>
<td>8</td>
<td>112</td>
</tr>
</tbody>
</table>

*Clotting tests from this date on were always normal.
thrombin time have been described elsewhere. Our one-stage quantitative proaccelerin assay has also been described elsewhere.

Our one-stage clotting system for measuring proconvertin consisted of: 0.2 ml. of proconvertin-deficient plasma (which supplied prothrombin, proaccelerin, Stuart factor and fibrinogen); 0.2 ml. of adsorbed ox plasma (an added source of proaccelerin and fibrinogen); 0.2 ml. of the plasma to be tested diluted 1:10 (which supplied the only source of proconvertin); 0.2 ml. of thromboplastin; and 0.2 ml. of 35 mM calcium chloride.

The two-stage prothrombin technic of Biggs and Macfarlane14 was modified as follows. The incubation mixture consisted of 0.4 ml. of brain extract thromboplastin, 0.4 ml. of undiluted test plasma, 0.1 ml. of calcium chloride, 0.4 ml. of 20 mM calcium chloride. Beginning at 15 seconds and 15 to 30 second intervals thereafter, 0.1 ml. of the incubation mixture was added to 0.4 ml. of a 100 mg. per cent bovine fibrinogen solution (Fibrinogen, Warner-Chilcott) in isotonic sodium chloride, and the clotting times were noted.

Our three-stage prothrombin assay was modeled after Stormorken’s.15 Thromboplastin, 0.2 ml., proconvertin reagent, 0.2 ml., accelerin reagent, 0.2 ml., 0.18 M pyrocatechol (to neutralize antithrombin),16 0.3 ml. and 30 mM calcium chloride solution, 0.2 ml., were mixed to make tissue prothrombinase. After three minutes, 0.2 ml. of a 1:10 dilution of the test material was added to the incubation mixture. One minute later and at successive half minutes until 3 minutes had elapsed, the thrombin activity in this final incubation mixture was measured by adding 0.2 ml. to 0.2 ml. of an adsorbed human plasma substrate which also contained a trace of pyrocatechol. The shortest clotting time so obtained was converted to prothrombin units from a thrombin-fibrinogen dilution curve prepared with the same substrate plasma. One prothrombin unit was defined as the amount of prothrombin in 0.2 ml. of the final incubation mixture which, when converted to thrombin, clotted the substrate plasma in 15 seconds at 37 C.

The thromboplastin generation test of Biggs and Macfarlane was used with substrate plasma mixtures as described later. Pool and Robinson’s technic of AHG assay was used.17 Antithrombin was measured as described by Biggs and Macfarlane.

Results

The following studies were undertaken to explain, (1) the long “prothrombin” times and (2) the mode of action of the anticoagulant which was found. The evidence for concluding that the long “prothrombin” times reflected true hypoprothrombinemia will be presented first. It may be summarized as follows:

1. Normal plasma shortened the patient’s long Quick “prothrombin” time.

The Quick clotting times of mixtures of the patient’s plasma with normal plasma and of mixtures of normal deprothrombinized plasma with normal plasma are listed in table 2. They show that a small amount of normal plasma shortened the Quick time of the patient’s plasma at least as effectively as it did that of deprothrombinized plasma.

As table 2 also shows, a minor effect of the anticoagulant was demonstrable when the mixtures contained equal parts or more of normal plasma. Such mixtures with the patient’s plasma took about a second longer to clot than those with deprothrombinized plasma. For example, on another occasion when the patient’s plasma clotted in 44 seconds, a mixture of equal parts of normal and patient’s plasma clotted in 19.6 seconds, whereas a mixture of equal parts of normal and normal absorbed plasma clotted in 18.0 seconds.

Thus, the major reason for the long Quick “prothrombin” time was a deficiency of a clotting factor supplied in vitro by normal plasma. Only a
Table 2.—A Comparison of the Quick “Prothrombin” Times of the Patient’s Plasma and of Normal Deprothrombinized Plasma Mixed with Varying Amounts of Normal Plasma

<table>
<thead>
<tr>
<th>Parts of</th>
<th>Parts of Pts. plasma</th>
<th>Deproth. normal plasma</th>
<th>Normal plasma</th>
<th>Quick time* (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>25.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>–</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>1</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>1 –</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>3 –</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>1 –</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>1 –</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>1 –</td>
<td>11.4</td>
<td></td>
</tr>
</tbody>
</table>

*Average of duplicate determinations.

Table 3.—The “Prothrombin” Activity in Patient J. G.’s Plasma as Measured in Various One-Stage “Prothrombin” Assays

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitive to variation in</th>
<th>Final dilution of test plasma</th>
<th>“Prothrombin” activity (% of normal) sample 1</th>
<th>sample 2</th>
<th>sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quick “Proth.” test</td>
<td>Prothrombin Proconvertin Stuart factor</td>
<td>1/3</td>
<td>21</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>Proth.-Proconv. test</td>
<td>Prothrombin Proconvertin Stuart factor</td>
<td>1:40</td>
<td>9</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Venom-“cephalin” proth. test</td>
<td>Prothrombin Stuart factor</td>
<td>1:160</td>
<td>8</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

small part of the prolongation could be attributed to an effect of the anticoagulant.

2. The abnormality in the “prothrombin” time increased with increasing specificity of the one-stage assay for prothrombin, despite the dilution of the test plasma required in the more specific assays.

The “prothrombin” times obtained with the Quick, prothrombin-proconvertin, and venom-“cephalin” methods are compared in table 3. The greatest defect was found in the venom-“cephalin” test, which is not only the most specific for prothrombin itself, but the assay in which the test plasma is diluted the most. If the long “prothrombin” times were due primarily to anticoagulant activity, we would have expected the greatest defect in the Quick test, which involves the least dilution of the test plasma.

Prothrombin was separated from the patient’s plasma by the barium citrate technic of Lewis and Ware. With this technic, prothrombin is found in dissolved Precipitate I. Dissolved Precipitate I from normal plasma gave values of 90 per cent in the prothrombin-proconvertin and 85 per cent in the
venom-“cephalin” assay. The patient’s dissolved Precipitate I gave values of 11 and 8 per cent, respectively, in these assays.

3. The patient’s plasma contained normal amounts of proaccelerin, proconvertin and Stuart factor.

Proaccelerin deficiency could not have caused the patient’s long prothrombin-proconvertin and venom-“cephalin” clotting times, for a reagent containing proaccelerin is added in these assays. Moreover, two samples of the patient’s plasma were found to contain 76 and 96 per cent proaccelerin, respectively, in a quantitative one-stage proaccelerin assay.

Proconvertin deficiency could not have caused the patient’s long venom-“cephalin” time, for this assay is independent of proconvertin. A 1/10 dilution of the patient’s plasma clotted in 26 seconds in our proconvertin assay as compared to 24 seconds and 76 seconds for normal plasma and control buffer, respectively. Thus, the patient’s plasma contained about as much proconvertin as normal plasma.

Either prothrombin or Stuart factor deficiency will prolong the venom-“cephalin” clotting time. However, the venom-“cephalin” time of a mixture of equal portions of the patient’s plasma and normal serum (which supplies Stuart factor but insignificant amounts of prothrombin) was still very long (194 seconds). In contrast, the venom-“cephalin” time of a mixture of equal portions of the patient’s plasma and normal plasma (which supplies adequate amounts of both Stuart factor and prothrombin) was shortened to 39 seconds, which was also the clotting time of a 50 per cent dilution of normal plasma in this assay. These mixing experiments strongly suggest that the patient’s venom-“cephalin” time resulted from true prothrombin deficiency without significant associated Stuart factor deficiency. We had no Stuart factor-deficient substrate plasma to measure the Stuart factor content of her plasma directly.

4. Prothrombin deficiency was found in two- and three-stage prothrombin assays.

Evidence suggesting a severe prothrombin deficiency was found in the two-stage assay of Biggs and Macfarlane. Normal plasma mixed with diluting fluid in the incubation mixture gave a minimum fibrinogen clotting time of 15 seconds. When the patient’s plasma was substituted for normal plasma, the minimum fibrinogen clotting time rose to 85 seconds. When the test was repeated with the patient’s plasma mixed with absorbed ox plasma (as an added source of proaccelerin) the minimum fibrinogen clotting time was 120 seconds.

Since the test plasma is diluted only about one-third in the incubation mixture, the failure to form a powerful thrombin in this test could reflect anticoagulant activity rather than hypoprothrombinemia. Therefore, the studies were repeated using diluted plasma in a three-stage assay. In this assay, potent tissue prothrombinase is made by incubating tissue thromboplastin with a reagent containing proconvertin and Stuart factor, a reagent containing accelerin and calcium. Then a 1/10 dilution of the test plasma is added to the incubation mixture and the thrombin generated is measured
Table 4.—Changes in the Proth.-Proconv. and Venom-“Cephalin” Tests Produced by Transfusing a Plasma Extract Rich in Prothrombin

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proth.-Proconv. test (%)</th>
<th>Venom-“Cephalin” test (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before extract</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>After extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediately</td>
<td>50</td>
<td>23</td>
</tr>
<tr>
<td>2 hours</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>4 hours</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>8 hours</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>22 hours</td>
<td>10</td>
<td>7</td>
</tr>
</tbody>
</table>

by clotting an adsorbed plasma substrate. The final dilution of the test plasma in our incubation mixture was 1/85.

Severe hypoprothrombinemia was also demonstrated with this technic. A normal plasma contained 701 prothrombin units per ml. The patient’s plasma contained less than 100 units per ml. The clotting times were too long to convert accurately to prothrombin units. A mixture of equal parts of the normal plasma and the patient’s plasma contained 374 prothrombin units per ml. The same value was obtained for a mixture of equal parts of the normal plasma and normal serum. By doubling this value and comparing it with the value for the normal plasma alone, we estimate that both the patient’s plasma and normal serum sample contained about 50 units of prothrombin per ml. These data clearly show that the failure to demonstrate prothrombin in the patient’s plasma was not due to an antiprothrombin interfering with the measurement of prothrombin in this assay.

5. The patient’s prothrombin level rose after the transfusion of prothrombin. It then fell at a steady rate to its original level.

The prothrombin-proconvertin and venom-“cephalin” times listed in table 4 were obtained before and at intervals after the transfusion, over 90 minutes, of 90 ml. of a plasma extract rich in prothrombin (made available through the kindness of Dr. Paul Aggeler). Note first that the values prior to the injection were higher than those obtained on the three previous days (listed in table 3) and those found when the effect of the extract had been dissipated. The patient had just finished receiving a blood transfusion when this initial sample was drawn. Thus, a single unit of blood produced a measurable rise in prothrombin activity. This would not have happened if the patient’s plasma had contained an antiprothrombin.

The extract further elevated prothrombin activity. The transfused prothrombin disappeared at an approximately steady rate over about eight hours. If an antiprothrombin had caused the long “prothrombin” times, we would have expected either no effect from the extract or a rise followed by a precipitous return to the original level.

This completes our evidence for the existence of true hypoprothrombinemia. Our remaining studies were concerned with the anticoagulant in the patient’s plasma and serum. They show that this anticoagulant interfered with both intrinsic clotting, i.e., clotting by blood prothrombinase, and extrinsic clotting, i.e., clotting by tissue prothrombinase. The experiments demonstrating
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interference with intrinsic clotting will be described first and may be summarized as follows:

1. *The patient's long partial thromboplastin time was not due to prothrombin deficiency alone.*

   In the partial thromboplastin time, “cephalin,” a reagent with platelet thromboplastic factor-like activity, is added to plasma. This makes the clotting time dependent only upon the plasma clotting factors, i.e., upon the initial rate of prothrombin conversion by blood prothrombinase.

   Our normal range for the partial thromboplastin time is 50 to 90 seconds. The patient’s plasma clotted in 187 seconds; a normal plasma clotted in 68 seconds. A mixture of one part of this normal plasma with nine parts of the patient’s plasma clotted in 177 seconds, whereas a mixture of one part of this normal plasma with nine parts of normal deprothrombinized plasma clotted in 110 seconds. A mixture of equal parts of this normal plasma and the patient’s plasma clotted in 121 seconds. In contrast, a normal clotting time of 65 seconds was obtained with a mixture of equal parts of this normal plasma and normal deprothrombinized plasma. These data indicate that the long partial thromboplastin time of the patient’s plasma was not primarily due to prothrombin deficiency.

2. *The patient’s plasma and serum lengthened the partial thromboplastin of normal plasma.*

   The partial thromboplastin time test was modified to make a clotting system consisting of 0.2 ml. of “cephalin” diluted 1/50, 0.2 ml. of normal plasma, 0.2 ml. of test material and 0.2 ml. of 30 mM calcium chloride. When buffer was used as the test material, the mixture clotted in 67 seconds. When a 1/5 dilution of normal serum was substituted for the buffer, the clotting time shortened to 59 seconds. However, when a 1/5 dilution of the patient’s plasma and of the patient’s serum were used as the test materials, the clotting times lengthened to 115 and 116 seconds, respectively.

   These data prove the presence of an anticoagulant in the patient’s plasma and serum. In later experiments using the same clotting system but patient’s plasma that had been stored frozen, we found that aluminum hydroxide adsorption did not remove the anticoagulant.

3. *The patient’s reagents formed a powerful blood prothrombinase in the incubation mixture of the “thromboplastin” generation test; however, clotting by blood prothrombinase was delayed when the patient’s plasma was used in a substrate plasma mixture.*

   The blood prothrombinase formed by mixing the patient’s platelets (400,000/cu.mm.), adsorbed plasma (diluted 1/5) and serum (diluted 1/10) in the incubation mixture of a thromboplastin generation test clotted a normal substrate plasma in 12 to 13 seconds. Under these conditions, the anticoagulant did not appear to interfere with blood prothrombinase generation.

   Because of its low prothrombin content, the patient’s plasma alone was not used as the substrate in the thromboplastin generation test. Instead, paired substrate mixtures, calculated to contain the same prothrombin concentration, were made by mixing varying proportions of normal and normal deprothrombinized plasma and by mixing varying proportions of normal
and patient's plasma. (The patient's plasma was assumed to contain no prothrombin.)

Blood prothrombinase was formed from normal platelets, the patient's adsorbed plasma, and the patient's serum and calcium. After five minutes of incubation, 0.2 ml. aliquots were taken from this mixture and added, with 0.2 ml. of 20 mM calcium chloride, to pairs of tubes containing 0.2 ml. of the substrate mixtures. Each pair of tubes contained the same concentration of prothrombin in the substrate but one tube was a normal-normal deprothrombinized plasma mixture and the other was a normal-patient's plasma mixture. After all of the pairs of substrate mixtures were clotted, the potency of the blood prothrombinase was rechecked with a normal plasma substrate.

The data from this experiment, listed in table 5, show that a longer clotting time was obtained at each prothrombin concentration for the substrate mixture containing the patient's plasma. The anticoagulant interfered with clotting by a formed, powerful blood prothrombinase.

4. The anticoagulant did not act like heparin.

Differences were found between the behavior of the patient's anticoagulant and heparin. Thus, our previous studies have shown\(^{11}\) that heparin would not lengthen the partial thromboplastin time when plasma or serum is diluted as in the experiments described above. Moreover, although the patient's plasma thrombin time was a little longer than normal, plasma from blood containing enough heparin to lengthen the Lee-White clotting time to 45 minutes does not clot at all in our thrombin time test.\(^{11}\) Finally, we could not abolish the anticoagulant's activity with protamine sulfate.

No difference was found between the antithrombin activity of the patient's serum and normal serum in the antithrombin test described by Biggs and Macfarlane.\(^{14}\)

Thus, in intrinsic clotting systems, we found an anticoagulant capable of interfering with clotting by formed blood prothrombinase but without heparin-like or antithrombic activity. The anticoagulant appeared to block prothrombin conversion by prothrombinase. This opinion was strengthened by our studies of the anticoagulant in extrinsic clotting systems, i.e., in clotting mixtures containing weak tissue thromboplastin or its products.

An explanation of our substrate mixtures is necessary before describing

**Table 5.—A Comparison of the Clotting Times in the “Thromboplastin” Generation Test of Substrate Plasma Mixtures Containing the Same Amount of Prothrombin but Made by Mixing Normal and Normal Adsorbed Plasma and by Mixing Normal and Patient’s Plasma**

<table>
<thead>
<tr>
<th>% Prothrombin in substrate mixture</th>
<th>Substrate clotting times (sec.)</th>
<th>Normal: normal ads.</th>
<th>Normal: pt.’s plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>9.6</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>11.2</td>
<td>13.8</td>
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<tr>
<td>25</td>
<td>15.0</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>18.0</td>
<td>21.6</td>
<td></td>
</tr>
</tbody>
</table>

Normal plasma alone

Beginning of exp. . . . . . . . 9.8
End of exp. . . . . . . . . . . 12.2
these experiments. Strong tissue thromboplastin clots normal plasma in 12 seconds by forming powerful tissue prothrombinase. Weak tissue thromboplastin takes much longer to clot normal plasma. Two reactions occur simultaneously. Some of the weak tissue thromboplastin forms weak tissue prothrombinase; the remainder, acting as a partial thromboplastin, initiates blood prothrombinase formation.  

This difference between the actions of strong and weak tissue thromboplastin is brought out with hemophilic plasma. Strong tissue thromboplastin clots normal and hemophilic plasma equally rapidly because clotting occurs within seconds only as a result of powerful tissue prothrombinase formation. In contrast, weak tissue thromboplastin clots normal plasma faster than it clots hemophilic plasma. In the normal plasma, the weak tissue thromboplastin can initiate both tissue and blood prothrombinase generation. In the hemophilic plasma, only tissue prothrombinase can form because blood prothrombinase generation requires antihemophilic globulin.

Therefore, we used hemophilic plasma to make substrate mixtures that would reflect the effect of the patient's anticoagulant upon extrinsic clotting only. One substrate mixture consisted of equal parts of Hemophilia A (AHG deficiency) plasma and normal serum; the other consisted of equal parts of Hemophilia A plasma and the patient's serum. Both contained the same content of prothrombin and proaccelerin (supplied by the Hemophilia A plasma) and of proconvertin and Stuart factor (supplied by both the Hemophilia A plasma and either the normal or the patient's serum). Blood prothrombinase could not form in either substrate mixture since neither Hemophilia A plasma nor serum contains antihemophilic globulin. Therefore, a longer clotting time in the substrate mixture containing the patient's serum could only mean interference with extrinsic clotting by the anticoagulant in the patient's serum.

Owren's theory of extrinsic clotting was followed. This theory states that when plasma containing tissue thromboplastin is recalcified, the tissue thromboplastin reacts first with proconvertin (and probably Stuart factor) to form convertin. Convertin is thought to convert enough prothrombin to thrombin to begin the activation of proaccelerin to accelerin. Convertin and accelerin then combine or act together to produce tissue prothrombinase activity, which is responsible for the rapid, massive conversion of prothrombin to thrombin. The evidence for these reactions has been presented in detail in Hjort's monograph.

Our experiments, designed to test the effect of the anticoagulant upon each of the above-cited steps, may be summarized as follows:

1. The anticoagulant interfered with clotting initiated by tissue thromboplastin.

Tissue thromboplastin was incubated with diluted, decalcified normal serum and 60 mM sodium chloride (in place of the calcium chloride used in later experiments). No preliminary reaction could occur during the incubation because calcium was absent. After three minutes' incubation, 0.2 ml of 20 mM calcium chloride and 0.2 ml of the incubation mixture (containing unchanged tissue thromboplastic activity) were added to 0.2 ml of the substrate mixtures and the clotting times were noted. As the data in the upper half of table 6 show, much longer times were found in the substrate mixture containing the patient's serum.

2. The anticoagulant interfered with clotting initiated by convertin.
Tissue thromboplastin was incubated with diluted, decalcified normal serum and 20 mM calcium chloride. In the presence of calcium, the tissue thromboplastin reacted with the proconvertin (and probably the Stuart factor) in the serum to form convertin. After three minutes' incubation, 0.2 ml of 20 mM calcium chloride and 0.2 ml of the incubation mixture (containing preformed convertin) were added to 0.2 ml of the substrate mixtures and the clotting times measured. These are listed in the bottom half of table 6. Note first that the clotting times in the bottom half of the table are shorter than the corresponding times in the top half. This is evidence that convertin activity did form in the incubation mixture. However, despite this preformation of convertin, much longer times were again found in the substrate mixture containing the patient's serum. Thus, the anticoagulant acted at a step in extrinsic clotting beyond convertin formation.

3. The anticoagulant did not interfere with the activation of proaccelerin to accelerin.

Bovine thrombin, 5 units in 0.1 ml., was added to 1 ml of the patient's plasma. Three minutes later, 0.1 ml of the thrombin-activated plasma was added to 0.9 ml of diluting fluid. After waiting 15 minutes to inactivate residual thrombin, we measured its accelerin content in the one-stage proaccelerin assay. The acceleration activity in the thrombin-activated plasma was almost six times that present in the untreated plasma.

In a second experiment, 2.0 ml of the patient's platelet-rich plasma was recalcified with 0.4 ml of 1 M calcium chloride. Aliquots were removed 1½ and 5 minutes after recalcification and tested for "thromboplastin" activity by addition to normal plasma with calcium; for thrombin by addition to adsorbed normal plasma without calcium; and for accelerin in the quantitative one-stage proaccelerin assay. Only traces of "thromboplastin" and thrombin formed during the five minutes after recalcification as shown by clotting times of over 100 and over 300 seconds for the unadsorbed and adsorbed substrate plasma, respectively. However, the accelerin activity five minutes after recalcification was almost four times greater than the proaccelerin activity of the plasma before recalcification.

These two experiments demonstrate that the anticoagulant did not interfere with the activation of proaccelerin to accelerin. The second experiment also illustrates that only minute quantities of thrombin are needed to begin the conversion of proaccelerin to accelerin.

4. The anticoagulant interfered with clotting initiated by tissue prothrombinase.

An incubation mixture was made consisting of 0.8 ml of tissue thromboplastin diluted 1/10, 0.2 ml of normal serum diluted 1/2.5 (source of proconvertin and Stuart factor), 0.4 ml of undiluted, thrombin-activated, adsorbed, normal plasma (source of ac-
COAGULATION DEFECT IN SYSTEMIC LUPUS ERYTHEMATOSUS

Celeryin) and 0.8 ml. of 20 mM calcium chloride. This mixture contained all of the clotting factors necessary to generate tissue prothrombinase activity. At the intervals after recalcification listed in table 7, 0.2 ml. of 20 mM calcium chloride and 0.2 ml. of the incubation mixture were added to the substrate mixtures and the clotting times noted. The experiment was then repeated, making a 1/10 dilution of the incubation mixture just before it was added to the substrate mixtures.

The clotting times are listed in table 7. With both undiluted and diluted tissue prothrombinase activity, longer clotting times were obtained in the substrate mixture containing the patient's serum. Thus, the anticoagulant was found to interfere with clotting in the presence of fully formed tissue prothrombinase activity.

**Discussion**

An antiprothrombinase ('antithromboplastin') blocking prothrombin conversion to thrombin by blood or tissue prothrombinase ('the formed thromboplastin complex') has been accepted as the primary plasma coagulation defect in systemic lupus erythematosus and lupus-like dysproteinemias. With one possible exception,21 every well studied patient with a plasma coagulation disturbance reported in the literature possessed this type of anticoagulant. The anticoagulant has not been found in other disorders.

Lupus has been described as an autoimmune disorder.24 The anticoagulant, which is a gamma globulin,25 has been thought of as an antibody against 'thromboplastin.'23,24,25

Frick3 drew attention to the association of the anticoagulant with false positive serology. We know of 14 instances of established lupus with an anticoagulant and recorded serologic studies—12 in the literature,1,5,24,27 a patient studied by Drs. Charles G. Craddock and Seymour Perry, and our own case. All except one4 have positive serology. This contrasts with an over-all estimate of 20 per cent incidence of false positive serology in lupus.28 Although differences exist between the antibody reacting with the phospholipids used in testing for syphilis and the lupus-anticoagulant,5,25 this very high incidence of positive serology in patients with the anticoagulant, and the presence of phospholipid in prothrombinase, suggest that the two phenomena are manifestations of closely related antibodies.

The difficulty in attributing all of the plasma coagulation defects to an antiprothrombinase antibody stems from an analysis of the "hypoprothrom-

Table 7.—Evidence that the Anticoagulant Interfered with Clotting Initiated by Preformed Tissue Prothrombinase

<table>
<thead>
<tr>
<th>Incubation product</th>
<th>Incubation time (min.)</th>
<th>Clotting times of substrate mixtures (sec.)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Hemo. A plasma plus normal serum</td>
</tr>
<tr>
<td>Tissue prothrombinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>undiluted</td>
<td>1</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.4</td>
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<tr>
<td></td>
<td>6</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>22.0</td>
</tr>
<tr>
<td>Tissue prothrombinase</td>
<td>1/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>40.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>37.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>55.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>48.7</td>
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binemia" which these patients exhibit. Hypoprothrombinemia defined as a prolonged Quick "prothrombin" time should exist, for the Quick test uses undiluted plasma which permits undiluted antiprothrombinase to interfere with prothrombin conversion. However, an antiprothrombinase cannot explain hypoprothrombinemia found with assays using dilute plasma nor the reduced prothrombin content found in a barium sulfate eluate free of anticoagulant activity. The latter observation led Bonnin and his co-workers to conclude that the lupus anticoagulant is an antiprothrombin rather than an antithromboplastin. Others have suggested that an antithromboplastin and an antiprothrombin may coexist. The possibility of true hypoprothrombinemia has also been recognized. Moreover, Loeliger has recently suggested that the hypoprothrombinemia develops because the anticoagulant binds prothrombin, which serves as its co-factor.

Our patient exhibited severe hypoprothrombinemia by every technic used to measure prothrombin. The hypoprothrombinemia could not be ascribed to the antiprothrombinase which she was also discovered to possess. Nor could we detect an antiprothrombin active against added prothrombin either in vitro or in vivo.

To explain her coagulation defects by a single autoimmune mechanism, we would have to postulate an antibody which could attach to her own but not to other people's prothrombin, and which, as a result, not only prevented the conversion of her prothrombin to thrombin but enhanced its ability to compete with normal prothrombin for prothrombinase. Even this contrived hypothesis breaks down, for a prothrombin adsorbent, aluminum hydroxide, failed to remove the anticoagulant activity from her plasma. The inability to absorb the lupus anticoagulant has been reported many times before. A double defect—true hypoprothrombinemia plus an antiprothrombinase—explains our data much more simply. It also explains puzzling features of previously reported cases, such as Frick's observation of constant hypoprothrombinemia, as measured by a two-stage method, in the face of a widely fluctuating Quick "prothrombin" time.

Many of the previously reported cases can be re-evaluated in terms of which defect predominated. The first case of Ley and his co-workers, like our own case, would fall at one extreme—with severe hypoprothrombinemia far overshadowing the manifestations of the antiprothrombinase. The boy with a severe hemorrhagic tendency, a prolonged clotting time and a prothrombin concentration of about 10 per cent mentioned by Harvey and his co-authors is most probably another example. At times hypoprothrombinemia may well have predominated in Frick's first case, in Ramot and Singer's patient and in the patient Dameshek described. In several cases, including our own, the hypoprothrombinemia was severe enough to suggest the possibility of congenital prothrombin deficiency.

The cases of Conley and Hartmann, Laurell and Nilsson, and Mueller and co-workers probably illustrate the other extreme—anticoagulant activity with little if any associated true prothrombin deficiency. Lee and Saunders' cases and Swift's patients may also fall into this group.

The patient described by Bonnin and co-workers, Loeliger's patient and
Frick's second and third patients illustrate the combination of anticoagulant activity with mild to moderate prothrombin deficiency. Further study might have placed more of the above-listed cases into this middle group.

The abnormal coagulation tests may be analyzed in terms of this dual mechanism. The long Lee-White clotting time, recalcification times and partial thromboplastin times primarily reflect anticoagulant activity. We infer this from the normal clotting times in glass tubes found in coumarin-induced hypoprothrombinemia and from the knowledge that the Lee-White test only detects severe deficiency states, below about 5 per cent of normal. The fact that the lupus-anticoagulant—interfering, as far as we know, only with prothrombin conversion by formed blood prothrombinase—lengthens the clotting time at all illustrates the dependence of blood prothrombinase generation, itself, upon prothrombin conversion.

The long Quick "prothrombin" time may be caused by anticoagulant activity, by hypoprothrombinemia, or by a mixture of the two. Therefore, to evaluate the Quick test, prothrombin must be measured with technics using diluted plasma. One must also determine whether or not normal plasma effectively shortens the Quick time.

We could partly relate the severity of bleeding to the presence of hypoprothrombinemia. As might be expected, severe hemorrhage occurred when the anticoagulant and severe hypoprothrombinemia coexisted, as in our own patient and in Ley's first case. In other patients with significant hypoprothrombinemia the bleeding tendency was less severe but apparently still the primary complaint leading to medical consultation. In contrast, several authors, whose patients we believe had little or no hypoprothrombinemia, commented upon the minimal evidence or even complete absence of clinical bleeding. However, this was not invariably so, for the patients of Laurell and Nilson appear to have had considerable difficulty with bleeding without significant hypoprothrombinemia. An associated thrombocytopenia contributed to the bleeding tendency in some patients.

We were impressed with the number of patients with gross hematuria. Only purpura exceeded gross hematuria in frequency. This brings to mind the characteristic glomerular capillary lesions of lupus, and suggests that vascular damage initiates the bleeding which the coagulation defect then aggravates.

Most patients were given adrenal steroid preparations, in varying amounts and with varying effectiveness. Our patient received large doses of prednisone orally for five days with no improvement. Within 24 hours of adding massive doses of intravenous hydrocortisone, her general condition improved remarkably and her bleeding ceased. Her coagulation tests returned to normal over about a two week period. We were impressed that her bleeding ceased before her coagulation tests improved.

Dameshek reported striking improvement in his patient's clinical and laboratory abnormalities within two months of beginning large doses of prednisone. Adrenal steroid therapy improved but did not correct the clotting and prothrombin times of Bonnin's patient. Ramot and Singer's patient...
exhibited marked clinical improvement on a maintenance dose of 75 to 100 mg. of cortisone daily, but no real change in his coagulation tests. Swift reported no change in his patient's laboratory tests on adrenal steroid therapy. However, some months later, after a steroid-induced remission had been achieved, her laboratory tests were normal.

Thus, it appears that adrenal steroids have no separate, specific effect upon the coagulation abnormalities. If the disease responds to adrenal steroids, the bleeding tendency subsides as part of the general clinical improvement. The coagulation defects diminish and may completely disappear, depending upon the extent of the remission obtained.

**Summary**

A patient has been described with systemic lupus erythematosus and severe bleeding. Her bleeding was associated with a complex plasma coagulation disturbance consisting of profound hypoprothrombinemia plus an anticoagulant active against formed blood and tissue prothrombinase. The problem of the recognition of hypoprothrombinemia in the presence of this type of anticoagulant has been discussed in detail.

An analysis of previously reported cases reveals that our patient's findings are not unique. It appears that the plasma coagulation disturbances of systemic lupus erythematosus characteristically result from a mixture of anticoagulant activity and true hypoprothrombinemia. In an individual patient one or the other may predominate.

**Summary in Interlingua**

Es describite un patiente con systemic lupus erythematose e grados sever de sanguination. Le sanguination esseva associate con un complexe disturbation coagulatori del plasma, consistente de marcate hypoprothrombinemia e, in plus, le presentia de un factor anticoagulante que ageva contra prothrombinase de sanguine formate e de histo. Le problema de recognoscer hypoprothrombinemia in le presentia de iste typo de anticoagulante es discutite in detalio.

Le analyse de previemente reportate casos revela que le constatationes in nostre patiente non es unic. Il pare que le disturbation coagulatori in le plasma de patientes con systemic lupus erythematose resulta characteristicamente ab un mixtura de activitate anticoagulante con ver hypoprothrombinemia. In le caso individual, le un o le altere del duo mentionate factores pote predominar.

**References**

6. Owren, P. A., and Aas, K.: The control of dicumarol therapy and the quanti-
COAGULATION DEFECT IN SYSTEMIC LUPUS ERYTHEMATOSUS


A Plasma Coagulation Defect in Systemic Lupus Erythematosus Arising from Hypoprothrombinemia Combined with Antiprothrombinase Activity

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