Studies on a Circulating Anticoagulant in Systemic Lupus Erythematosus: Evidence for Inhibition of the Function of Activated Plasma Thromboplastin Antecedent (Factor XIa)

By Henry Krieger, John P. Leddy, and Robert T. Breckenridge

Plasma proteins which interfere with blood coagulation have often been described in patients with systemic lupus erythematosus (SLE). The most frequent type interferes with the conversion of prothrombin to thrombin and thus prolongs the prothrombin time. Infrequently, SLE patients exhibit anticoagulants which appear to block the earlier stages of coagulation such as those involving factor VIII or the formation of activated factor XI (factor Xla). The anticoagulant reported here was studied by means of a sequential clotting system utilizing crude coagulation factors and was noted to interfere with the action of activated plasma thromboplastin antecedent (PTA) during the activation of factor IX. This anticoagulant was found in γ-globulin-rich ethanol fractions of plasma. After gel filtration, it was found principally in fractions containing IgM globulins but also, to a lesser extent, in IgG-rich fractions. In this respect, it is similar to anticoagulants reported in certain other cases of SLE. Attempts to confirm the immunoglobulin nature of the anticoagulant by immune-absorption were, however, inconclusive.

The presence of proteins which interfere with blood coagulation (circulating anticoagulants) in the plasma of patients with systemic lupus erythematosus (SLE) or lupus-like syndromes have been reported by a number of investigators. The most frequently reported anticoagulant interferes with the conversion of prothrombin to thrombin either by blocking the interaction of Stuart factor (factor X) and proaccelerin (factor V) or by interfering with the subsequent conversion of prothrombin. Occasionally, patients are described with acquired hemophilia due to an anti-factor VIII-type anticoagulant during the course of SLE. Recently, patients with anticoagulants which apparently interfere with an earlier stage of coagulation have been reported, although the exact site of action is not always clear.

We have recently had the opportunity to study a patient with systemic lupus erythematosus who demonstrated a prolonged partial thromboplastin time and a normal prothrombin time. By means of a sequential clotting system utilizing crude coagulation factors it was possible to demonstrate that this patient's anticoagulant inhibited the action of activated plasma thromboplastin antecedent (factor Xla) during the conversion of Christmas factor (factor IX) to its active form.
Table 1. Coagulation Studies

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time</td>
<td>16 sec</td>
<td>15 sec</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>21 sec</td>
<td>18 sec</td>
</tr>
<tr>
<td>Activated partial thromboplastin time</td>
<td>64 sec</td>
<td>33 sec</td>
</tr>
<tr>
<td>1:1 Mix of patient and normal*</td>
<td>63 sec</td>
<td></td>
</tr>
<tr>
<td>Factor IX</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Factor VIII</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Factor XI</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>Factor XII</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Factor XII</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

*In this test the patient's plasma was mixed 1:1 (v/v) with normal plasma and then an activated partial thromboplastin time done on the mixture.

form (factor IXa). These studies confirm the report of Castro et al.\(^9\) and enlarge upon their observations of this unusual inhibitor.

CASE REPORT

VA is a 56-yr-old white female who was admitted to the Rochester General Hospital in March 1973 with the complaints of fatigue and jaundice.

She had previously been admitted in December 1972 following an automobile accident during which she sustained a retroperitoneal hemorrhage from an angiomyolipoma of the kidney. The kidney was subsequently removed. No coagulation studies other than a normal platelet count and a normal prothrombin time were obtained prior to surgery, but there were no hemorrhagic complications, and she was discharged on postoperative day 10.

During the March admission, jaundice (bilirubin, 10.5 mg/dl) and hepatosplenomegaly were present. Laboratory data included: hematocrits of 34-36%, 5% reticulocytes, negative Coombs' test, white cell count of 3000/cu mm, normal platelets, and negative tests for occult blood in the stool. The antinuclear antibody (ANA) was positive in a titer of 1:128, although the LE cell test was negative. Antibodies to both single-stranded and native DNA were elevated, by radioimmunoassay, and this finding, plus the presence of glomerular changes in the removed kidney compatible with SLE, confirmed the clinical impression.

Because of the somewhat obscure nature of this patient’s liver disease, in that she was an alcoholic who had had blood transfusions during her previous surgery, a liver biopsy was proposed, and screening coagulation studies were ordered. The PTT was 90 sec (control, 35 sec), and the prothrombin time was normal (see Table 1). When tests for the presence of a circulating anticoagulant were positive, the biopsy was cancelled.

During the ensuing month, her jaundice cleared without therapy, and although the hemolytic anemia and leukopenia persisted, she was discharged. She has remained compensated, without hemorrhagic symptoms, during the ensuing 30 mo. Recently (November 1974) we had another opportunity to study her plasma for the presence of a circulating anticoagulant. At that time the anticoagulant was no longer detectable.

MATERIALS AND METHODS

Blood was collected in uncoated plastic tubes using 3.8% sodium citrate, pH 5.0, as the anticoagulant, and the plasma was prepared as previously described.\(^11\) Sodium oxalate (0.1 M) was used as anticoagulant for experiments in which adsorption of the plasma with calcium phosphate was performed. The plasmas were used immediately or stored at -20°C until needed. All dilutions were performed with barbital-saline buffer, pH 7.5, unless otherwise noted.

Gliddex "P", a phospholipid mixture derived from soybeans, was prepared as described previously, as was Russell’s viper venom (Stypven).\(^11\)
All coagulation studies were performed in 12 × 75-mm disposable glass tubes according to methods previously published.\textsuperscript{1,12} Specific coagulation factor assays were performed by published techniques utilizing as substrates plasma deficient in the coagulation factor being tested.

Crude Christmas factor (Factor IX) was prepared from the plasma of a patient with severe classic hemophilia (factor VIII deficiency). The oxalated plasma was adsorbed with 10 mg/ml calcium phosphate for 10 min at room temperature, with constant stirring. After centrifugation, the precipitate was washed three times with cold isotonic saline and then eluted with 3.8% sodium citrate. The supernatant was stored at −20°C until used. This preparation was rich in Christmas factor, Stuart factor (factor X), prothrombin (factor II), and factor VII (proSPCA). It did not contain thrombin or other activated clotting factors, since it would not shorten the unactivated partial thromboplastin time of normal plasma.

Crude activated plasma thromboplastin antecedent (PTA) was prepared according to the method of Amir et al.\textsuperscript{13} This celite-activated PTA could not be further activated by contact with kaolin. It was stored at −20°C until used.

\textbf{Preparation of the Anticoagulant}

Plasma was obtained from the patient and a normal control in 0.13 M buffered sodium citrate. The vitamin K-dependent clotting factors were removed by adsorption with aluminum hydroxide gel (Amphogel, Wyeth Laboratories, Philadelphia, Pa.) and subsequent centrifugation. The supernatant plasma was then heated at 56°C for 30 min. In this step, fibrinogen was precipitated and the residual proaccelerin and antihemophilic factor were inactivated along with any remaining traces of vitamin K-dependent clotting factors.

The supernatant was mixed with an equal volume of 0.15 M sodium acetate (adjusted to pH 5.2 with 1 M acetic acid). Two volumes of deionized water and 75 mg of carboxymethylcellulose (CM Cation Exchanger, 0.6 meq/g, Sigma Chemical Co. St. Louis, Mo.) were added per ml of supernatant. This mixture was stirred constantly for 10 min at room temperature and subsequently centrifuged. The resultant supernatant, devoid of recognized coagulation factors, was dialyzed against barbital saline buffer at 4°C. After dialysis, the supernatant was brought to a final concentration of 25% ethanol by the addition of cold ethanol at −5°C. After 15 min of constant stirring, the mixture was centrifuged and the precipitate suspended in normal saline. This material, after dialysis against barbital saline, served as a source of the anticoagulant for the experiments to be described. Normal plasma treated in a similar fashion served as the control.

The anticoagulant in a given preparation was quantified by assuming that the patient’s untreated plasma contained 1 U of anticoagulant per ml. It was then possible to express the amount of anticoagulant present in either the crude ethanol fraction or the fractions prepared by gel filtration. During the partial purification of the anticoagulant, losses of activity occurred. For example, in one experiment in which the crude ethanol fraction was subjected to gel filtration on Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), the crude fraction contained 0.2 U of anticoagulant per ml, the IgG fraction contained 0.01 U/ml, and the IgM fraction contained 0.03 U/ml.

\textbf{Effects of the Anticoagulant}

\textit{The effect of the anticoagulant on the earlier stages of coagulation.} This was tested by adding 0.1 ml of the celite-activated PTA to 0.2 ml of normal platelet-deficient plasma. 0.1 ml of Gliddex was then added and the mixture agitated. At this point 0.1 ml of the anticoagulant fraction (or control) was added, followed by 0.1 ml 0.05 M calcium chloride. The clotting time of this mixture was then determined. By means of this procedure, if the anticoagulant interfered with the action of Hageman factor only, presumably the presence of the activated PTA in the mixture would circumvent that step, and the clotting time would be normal. Alternatively if the anticoagulant worked at a later step, it would prolong the clotting time of this mixture.

\textit{The effect of the anticoagulant on activated PTA.} This effect was tested in the following manner: 0.1 ml of the celite-activated PTA and 0.1 ml of the anticoagulant (or control) fraction were incubated in a 10 × 75-mm plastic tube at 37°C. At various intervals, 0.1 ml of this mixture was removed and immediately added to a mixture of 0.2 ml PTA-deficient plasma and 0.1 ml Gliddex. After agitation, 0.1 ml 0.05 M CaCl was added and the clotting time determined.
The inhibitory effect of the anticoagulant fraction was quantified by comparing its clotting time to a dilution curve of the control experiment. Thus the results could then be expressed in terms of the clotting time and the per cent of factor Xa inhibited.

The effect of the anticoagulant on the interaction between activated PTA and Christmas factor. This was tested in the following manner: 0.1 ml of the celite-activated PTA, 0.2 ml of crude Christmas factor, 0.1 ml of buffer, 0.1 ml of 0.05 M CaCl₂, and either 0.1 ml of anticoagulant or control fraction were incubated in a 10 × 75-mm plastic test tube at 37°C. At intervals, 0.1 ml of this mixture was diluted 1:10 in citrated barbital saline and added immediately to a mixture of 0.1 ml Christmas factor-deficient plasma, 0.1 ml Gliddex, and 0.1 ml buffer. This mixture was then agitated, 0.1 ml 0.05 M CaCl₂ added, and a clotting time determined. If the anticoagulant was active against either activated PTA or Christmas factor, the clotting time of this experiment would be prolonged.

Again, the inhibitory effect of the anticoagulant fraction was quantified by comparing the clotting time of the mixture to a dilution curve of the control experiment at 35 min (see Table 4). The results obtained with the anticoagulant fraction were then expressed both as a clotting time and the percentage of inhibition of the generation of activated Christmas factor.

The effect of the anticoagulant against activated Christmas factor. This effect was tested in the manner previously reported from this laboratory. In this experiment, after Christmas factor was fully activated, the anticoagulant (or control) fraction was added and the subsequent effect on the clotting time of factor IX-deficient plasma noted. The results were quantified by comparing the clotting times generated in the presence of the anticoagulant fraction to a dilution curve of the control experiment. The inhibition was then expressed as a clotting time and the per cent of active PTA inhibited.

Gel Filtration

In many experiments the crude, cold ethanol preparation or whole plasma from the patient and from normal controls were fractionated by gel filtration of Sephadex G-200. Each column had been carefully calibrated by prior fractionation of normal serum containing 125I-IgG myeloma protein as a marker. One milliliter plasma or 2-3 ml crude fraction were applied to 2.5 × 90-cm columns in borate-saline buffer, pH 8.0, and eluted at a flow rate of 8 ml/hr. Unconcentrated fractions did not consistently exhibit anticoagulant activity. Therefore, guided by the optical density profile of protein elution and by careful immunodiffusion analysis with specific antisera to IgM, IgA, and IgG, fractions from various peaks or regions were pooled and concentrated by pervaporation. When whole plasma was the starting material, four concentrated pools were regularly obtained: a high-molecular-weight or IgM-rich pool, an IgA-rich pool, an IgG-rich pool, and an albumin pool. When the crude ethanol preparation was the starting material, just two peaks were obtained: IgM rich and IgG rich, respectively. After dialysis against barbital-saline buffer, the concentrated pools were tested for anticoagulant activity by their ability to prolong the activated partial thromboplastin time (APTT) of normal plasma.

Immunoabsorption

Rabbit antisera to human IgM, IgG, IgA, or IgD were prepared in our laboratory against purified proteins. Specificity was achieved by stepwise absorption (to slight antigen excess) with immunoglobulins of the other classes, with light chains of both types, and with agammaglobulinemic serum. Rabbit antiserum to bovine albumin (anti-BSA) was kindly supplied by Dr. P. Z. Allen, Department of Microbiology. A weak cross-reactivity with human albumin was removed by absorption. Each anti-Ig serum was monospecific by immunoelectrophoresis, Ouchterlony analysis, and hemagglutination reactions. For the experiments below, portions of these antisera were heated at 56°C for 30 min to destroy procoagulant activity.

These antisera were used in attempts to block or remove the patient’s anticoagulant activity. Most experiments were done with the IgM-rich pool eluted from Sephadex G-200, since this material exhibited the highest and most consistent anticoagulant activity of any isolated fraction. In one experiment the crude, cold ethanol preparation served as the source of anticoagulant. Multiple 250-μl aliquots of corresponding fractions from patient and control were mixed with 125 μl of each antiserum. The latter were usually undiluted, but in some experiments 1:2, 1:5,
ANTICOAGULANT IN SLE

Table 2. Experiments to Determine if the Anticoagulant Interfered With the Conversion of Prothrombin to Thrombin or Fibrinogen to Fibrin.*

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Activated Partial Thromboplastin Time (sec)</th>
<th>Stypven Time (1:80,000) (sec)</th>
<th>Thrombin Time (5 U/ml) (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasma</td>
<td>48.0</td>
<td>23.0</td>
<td>14.9</td>
</tr>
<tr>
<td>Normal plasma + anticoagulant (1:1)</td>
<td>95.0</td>
<td>22.2</td>
<td>20.2</td>
</tr>
<tr>
<td>Normal plasma + control (1:1)</td>
<td>53.3</td>
<td>20.0</td>
<td>19.4</td>
</tr>
</tbody>
</table>

*In this set of experiments the anticoagulant containing plasma and the control were treated in an identical fashion (see Materials and Methods).

and 1:10 dilutions were employed. After incubation at 37°C for 60 min and at 4°C overnight, the mixtures were spun at 2000 rpm for 60 min in a PR-J International Centrifuge to remove any precipitates. Precipitates were observed regularly when anti-IgM was reacted with either the IgM-rich Sephadex pool or the crude ethanol fraction. Supernatants of all reaction mixtures were then tested in parallel for anticoagulant activity by activated PTT assay and compared to aliquots which had been reacted only with saline.

RESULTS

The patient was noted to have a prolonged PTT with a normal prothrombin time (Table 1), and when her plasma was mixed with normal plasma, it prolonged the PTT of the normal: presumptive evidence of the presence of an anticoagulant. Unlike the usual anticoagulants in SLE, this inhibitor did not affect the conversion of prothrombin to thrombin or subsequent effect of thrombin on fibrinogen (Table 2).

This anticoagulant manifested its action after the activation of PTA, since it prolonged the clotting time of mixtures after PTA was maximally activated (Table 3). It can also be seen from Table 3 that 75% of the activated PTA generated during this experiment was inhibited by the anticoagulant fraction.

The anticoagulant's effect upon the interaction of activated PTA and Christmas factor is noted in Table 4. It can be seen from this experiment that the anticoagulant interferes with the interaction of activated PTA and Christmas factor throughout the period tested in that only 60% of the Christmas factor is activated in the presence of the inhibitor. We have no explanation for the consistent finding of increased inhibition of the clotting times with incubation. It is conceivable that this anticoagulant only manifests its action once the Hageman factor (factor XII) has been activated, but this is only speculative. It can also

Table 3. Experiments to Determine if the Anticoagulant Manifested its Inhibition After the Activation of PTA*

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Clotting Time (sec)</th>
<th>Active PTA Inhibited (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active PTA + normal plasma + anticoagulant</td>
<td>106.9</td>
<td>75</td>
</tr>
<tr>
<td>Active PTA + normal plasma + control</td>
<td>72.4</td>
<td></td>
</tr>
</tbody>
</table>

*0.1 ml of celite-activated PTA, diluted 1:10, was mixed with 0.2 ml normal platelet-poor plasma. Either 0.1 ml of the anticoagulant fraction (or the normal fraction) was then added and the mixture agitated. 0.1 ml Gliddex and 0.1 ml 0.05 M CaCl₂ were then added and the clotting time determined. The results were quantified as noted in the text and expressed both as a clotting time and the per cent of factor X1a inhibited.
Table 4. Effect of the Anticoagulant on the Reaction Between Activated PTA and Christmas Factor

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Incubation Time (min)</th>
<th>Clotting Time (sec)</th>
<th>Factor IXa Generated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticoagulant</td>
<td>0</td>
<td>438</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>266</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>116</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>68</td>
<td>64</td>
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<tr>
<td></td>
<td>48</td>
<td>99</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>417</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>126</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>71</td>
<td>60</td>
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<td>56</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>117</td>
<td>—</td>
</tr>
</tbody>
</table>

*0.1 ml of celite-activated PTA, 0.2 ml Christmas factor, 0.1 ml buffer, 0.1 ml of 0.05 M CaCl₂, and 0.1 ml of anticoagulant (or control) were mixed and incubated at 37°C. At the intervals noted, 0.1 ml of the mixture was diluted 1:10 in citrated buffer and 0.1 ml added to a mixture of 0.1 ml Christmas-deficient plasma, 0.1 ml Gliddex, and 0.1 ml buffer. 0.1 ml of 0.05 M CaCl₂ was then added and the clotting time determined. The results were quantified as noted in the text and expressed both as a clotting time and as a per cent of factor IXa generated.

be noted that an unstable product is formed by 35 min which deteriorates with prolonged incubation (presumably activated Christmas factor).

Since the anticoagulant appeared to interfere with coagulation after the activation of PTA and before the conversion of prothrombin to thrombin, experiments were done to determine if it was inhibitory once Christmas factor was maximally activated. Activated PTA and Christmas factor were incubated together until maximum development of factor IXa was noted (35 min). At this time, no difference between the inhibitor and a similar fraction of normal plasma could be detected (Table 5). This finding suggested that the anticoagulant was no longer inhibitory once factor IXa was formed.

The anticoagulant's inhibitory properties against active PTA were immediate, since the anticoagulant inhibited 70%-75% of the activated PTA without pre-incubation (Table 6). It would also appear that activated PTA may be unstable in the presence of this inhibitor (note results at 45 min, Table 6).

The inhibitory action of the anticoagulant could be overcome by increasing the amount of PTA in a mixture of normal plasma and anticoagulant. In this experiment the activated PTA preparation was prepared as noted in Materials and Methods. It was then added to normal plasma, Gliddex, anticoagulant (or normal), and calcium in plastic tubes and the clotting time recorded. When the

Table 5. Effect of Anticoagulant After Christmas Factor Had Been Activated

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Clotting Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Christmas factor + anticoagulant</td>
<td>140</td>
</tr>
<tr>
<td>Active Christmas factor + control</td>
<td>139</td>
</tr>
</tbody>
</table>

*In this experiment, 0.1 ml of celite-activated PTA, 0.2 ml Christmas factor, 0.1 ml buffer, and 0.1 ml 0.05 M CaCl₂ were mixed and incubated at 37°C for 35 min. 0.1 ml of the mixture was then diluted 1:10 in citrated buffer and added to Christmas-deficient plasma. 0.1 ml of anticoagulant (or control) was added along with 0.1 ml Gliddex. 0.1 ml of 0.05 M CaCl₂ was added and a clotting time determined.
## Table 6. Effect of Anticoagulant on Activated PTA*

<table>
<thead>
<tr>
<th>Incubation Time (min)</th>
<th>Clotting Time (sec)</th>
<th>Activated PTA Inhibited (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticoagulant fraction + activated PTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>135</td>
<td>70</td>
</tr>
<tr>
<td>15</td>
<td>155</td>
<td>75</td>
</tr>
<tr>
<td>30</td>
<td>150</td>
<td>75</td>
</tr>
<tr>
<td>45</td>
<td>180</td>
<td>90</td>
</tr>
<tr>
<td>Control fraction + activated PTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>113</td>
<td></td>
</tr>
</tbody>
</table>

*In this experiment, 0.1 ml of celite-activated PTA and 0.1 ml of anticoagulant (or control) were mixed and incubated for the times shown. At the end of these intervals, the mixtures were tested for their ability to shorten the clotting time of PTA-deficient plasma. The results were quantified as noted in the text and expressed as both a clotting time and the per cent of activated PTA inhibited.

Concentration of activated PTA was raised to ten times the concentration present in normal plasma, the inhibitory activity of the anticoagulant fraction was no longer detectable, since the clotting time of this mixture containing the anticoagulant fraction was identical to that of the mixture containing the normal fraction.

Fractionation of the patient's plasma, or of the crude ethanol preparation, on Sephadex G-200 columns revealed that anticoagulant activity was most often and most prominently associated with IgM globulins in the first (high-molecular-weight) peak. In some experiments, lesser amounts of activity appeared in the IgG-rich peak and, when plasma was the starting material, also in the IgA-rich region between the IgM and IgG peaks. Such secondary areas of activity were found often enough, particularly in respect to the IgG-rich fractions, that they could not be dismissed as IgM contamination. Indeed, the separation of IgM and IgG in such runs was complete, as determined by immunodiffusion analysis of the concentrated pools and by the narrow elution zone of the $^{125}$I-IgG marker. More confident interpretation of these zones of lesser activity could probably have been made if the patient's anticoagulant had been present in higher titer. In any event, no anticoagulant activity was found in any fraction from normal controls studied concurrently.

Attempts to clarify this issue by immunoabsorption with specific antisera (see Materials and Methods) were inconclusive. For example, consistent and clear-cut reduction of anticoagulant activity was not achieved with potent anti-IgM. Two problems impeded such studies: (1) seemingly nonspecific reduction of anticoagulant activity in some experiments by control antisera such as anti-BSA or anti-IgD and (2) apparent lability of the anticoagulant after Sephadex fractionation.

**DISCUSSION**

Circulating anticoagulants have frequently been described in systemic lupus erythematosus (SLE) since the first report by Conley and Hartman. In fact, in a large series reported by Margolius et al. this complication was recognized in 13% of patients with SLE. The most frequent anticoagulant reported has
manifested its action during the conversion of prothrombin to thrombin, and, because of the associated finding of hypoprothrombinemia in some patients, antiprothrombin activity has been postulated by several investigators. Others have described antithrombin activity. Before 1962, anticoagulants in lupus interfering with the formation of intrinsic thromboplastin were suggested but not well documented. In 1962, Breckenridge and Ratnoff described anticoagulants which appeared to interfere with the reaction between activated Stuart factor (factor X) and proaccelerin (factor V). Subsequently, anticoagulants directed against other coagulation proteins involved in the formation of the intrinsic prothrombin-converting principal have been described, including those which apparently inhibited PTA (factor XI) or Hageman factor (factor XII). Indeed, Castro and his co-workers felt that their studies demonstrated interference with the interaction of factors XIa and IX in one of their patients, and Biggs and Denson have described interference with the interaction between factor IX and factor VIII.

The studies reported here confirm the report of Castro and his colleagues in the demonstration that occasional anticoagulants interfere with the interaction of activated PTA and Christmas factor. Unlike the inhibitor described by Biggs and Denson, however, this anticoagulant was no longer inhibitory once Christmas factor was maximally activated.

The type of protein responsible for our patient’s inhibitor was not fully clarified. Anticoagulant activity was associated with a γ-globulin-rich fraction of plasma obtained by cold ethanol fractionation. By gel filtration, this activity was found principally in the high-molecular-weight (IgM-rich) peak but also to a lesser extent in association with IgG globulins. The anticoagulant activity in plasma, therefore, could represent a mixture of IgM and IgG antibodies, as reported by others. On the other hand, these studies do not prove that the anticoagulant proteins are immunoglobins. The unexpected difficulty in neutralizing this anticoagulant by specific antisera to IgM or IgG, its apparent lability after gel filtration, and its somewhat variable appearance in different Sephadex fractions leave open the possibility of a nonimmunologic inhibitor subject to breakdown or cleavage.

The occurrence of an anticoagulant directed against PTA is unusual. Although such inhibitors have been noted in patients with congenital deficiencies of PTA and in normal plasma, the inhibitor reported here is different from these in its molecular characteristics and did not arise in a patient with a hereditary hemorrhagic disease.

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