Acquired Circulating Anticoagulants in Systemic
“Collagen Disease”

Auto-immune Thromboplastin Deficiency

By Paul G. Frick

With the technical assistance of Mary K. Weimer

INTRODUCTION

The occurrence of circulating anticoagulants in lupus erythematosus disseminatus and related conditions conventionally grouped under the term “collagen disease” is rather rare. Conley reported two cases of lupus erythematosus with clotting inhibitors in 1952, one of which he had already described in 1948. Hitzig reported a case of Libman-Sacks syndrome in which the anticoagulant was present only temporarily. Ley and co-workers reported a case of idiopathic hypoprothrombinemia with arthralgia, albuminuria, positive serology with postmortem kidney changes which were indistinguishable from the “wire loop” lesions of disseminated lupus erythematosus. The failure of the prolonged prothrombin time to respond to blood transfusions strongly suggests that the patient had a circulating anticoagulant. The multisystem disease characterizing a patient with a circulating anticoagulant and thrombocytopenia reported by Barkham justifies the classification of this case as “collagen disease,” even though the author did not specifically use this term. An additional case with similar abnormalities has recently been published by Nilsson and Wenekert.

This report concerns three cases of “collagen disease” with acquired circulating anticoagulants. The first case was a woman whose clinical, laboratory and autopsy findings were typical for lupus erythematosus disseminatus, but in whom it has never been possible to demonstrate typical L.E. cells. She gave birth to an infant in whom the anticoagulant was demonstrated over a period of seven weeks after birth. The second patient developed the anticoagulant while under our own observation after a drug reaction. The third patient had lupus erythematosus disseminatus with hemolytic anemia; this is the only case where typical L.E. cells were found.

MATERIAL AND METHODS

Collection and processing of blood, plasma, and serum. Silicone coated syringes, test tubes and pipettes were used for all experiments except for the determination of the venous clotting time and the prothrombin consumption test which were performed in plain glass tubes. The needles were treated with Arquad 2C. Plasma was prepared with 0.1M sodium oxalate or 3.2 per cent sodium citrate solution, depending on the test to be performed. All blood samples were mixed with 1/10 volume of anticoagulant.

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BaSO₄ plasma was prepared by adding 100 mg. of BaSO₄ (Merck & Co., Rahway, N. J.) to 1.0 ml. of oxalated plasma. The mixture was gently shaken for 5 minutes and BaSO₄ removed by centrifugation (2000 rpm for 10 minutes).

48 hour old serum used as source of stable prothrombin conversion factor was obtained by centrifuging normal blood collected under sterile conditions and left at 37 C. for 48 hours in plain glass tubes.

Labile factor deficient plasma was obtained by storing normal oxalated plasma at 20-24 C. for 10-12 days under sterile conditions.

Stable factor deficient plasma was obtained from a patient with a congenital deficiency of this factor. 

Difco rabbit brain thromboplastin was used throughout.

Armour's bovine fraction I served as fibrinogen. One hundred mg. of dry powder were dissolved in 9.5 ml. of 0.9 per cent NaCl solution and 0.5 ml. of 1.72 per cent imidazole buffer with a pH of 7.2.

Bleeding time. Method of Ivy. 

Clotting time of venous blood. Modified Lee White method: One ml. of blood was placed in each of two glass tubes (75 x 8 mm.) which were tilted every minute. The first tube was started 10 minutes after venipuncture. The second one was started when the endpoint of the first was reached. The endpoint of the second tube was recorded as clotting time of venous blood.


Prothrombin time. One-stage method of Quick.

Recalcification time. Determination of the time interval between the addition of 0.1 ml. of 1/40M CaCl₂ solution to 0.1 ml. of citrated plasma and the appearance of a clot.

Platelets. Method of Rees and Ecker.

Capillary fragility. Rumpel-Leede test.

Prothrombin determination in units per ml. Modification of the two-stage method of Ware and Seegers. Citrated plasma was not defibrinated and the addition of BaCO₃ treated bovine serum as a source of Ac-globulin was omitted.

Fibrinogen determination. Precipitation of fibrin according to the method of Cullen and Van Slyke and micro-Kjeldahl nitrogen determination on the fibrin clot obtained from 0.1 ml. of plasma with the method of Ma and Zuazaga.

Prothrombin consumption test. Blood was collected in a siliconized syringe and distributed into a series of dry glass tubes (75 x 8 mm.) calibrated at 2 ml. All tubes were placed in a water-bath at 37 C. At 20 minute intervals for the first hour, and at 30 minute intervals for the second hour, 0.2 ml. of 3.2 per cent sodium citrate solution were added to successive tubes. The mixture was stirred with a glass rod and the serum obtained by centrifugation (2000 rpm for five minutes). A one-stage prothrombin time and a two-stage determination of prothrombin concentration were then performed on each sample of serum.

Thrombin clotting times. Determination of the time interval between the addition of 0.1 ml. of various dilutions of thrombin solution to 0.1 ml. of oxalated plasma and the appearance of a clot. The highest concentration of thrombin solution gave a clotting time of 10 seconds with normal plasma. Commercial bovine thrombin (Parke, Davis & Co., Detroit, Michigan) was used for all experiments.

Protamine titration. One tenth ml. of various concentrations of protamine sulfate solution ranging between 5 and 100 mg. per cent were added to 0.9 ml. of citrated plasma. A one-stage prothrombin time and recalcification time were performed on the various mixtures and compared with a control in which 0.1 ml. of 0.9 per cent NaCl solution was used instead of protamine.

Thromboplastin titration. One-stage prothrombin times with various dilutions of thromboplastin. The undiluted thromboplastin solution was prepared by incubating 150 mg. of Difco thromboplastin in 4.0 ml. of 0.9 per cent NaCl solution at 48 C. for 10 minutes.

Case Reports

Case 1. A 36 year old housewife, who has been followed at the University of Minnesota Hospitals for a period of four years, originally noticed menorrhagia and subcutaneous
hematomas after minor trauma since the delivery of her first child in 1946. She was found
to have a positive serology (Wassermann) during the last trimester of her first pregnancy,
while six months prior to conception it was negative. Since the infant at age one and the
husband had a negative Wassermann test, it was assumed that one was dealing with a false
positive serologic test. During the course of the patient's second pregnancy in 1948,
she developed a nephrotic syndrome of undetermined etiology. The patient delivered her
third child on December 24, 1951. The postpartum blood loss was rather copious this time.
In 1950 the patient was found to have a circulating anticoagulant which was also present
in the blood of the third baby during the first seven weeks of life. Past history includes a
tonsillectomy at age 8 and an appendectomy at age 19 without hemorrhagic complications.
There was no family history of hemorrhagic diseases.

Final admission: On May 31, 1954, the patient developed acute abdominal pain fol-
lowed by a paralytic ileus. Despite continuous suction with intestinal decompression
tubes and administration of blood, plasma, glucose, electrolytes and antibiotics the patient
expired on June 13. The autopsy revealed scattered intramural hemorrhages throughout
the wall of the small bowel. Both adrenals were replaced by large hematomas. The surface
of both kidneys was studded with pin point hemorrhages. On histologic examination,
the majority of the small vessels of the internal organs revealed fibrinoid deposits in
their wall. The glomeruli showed typical "wire loop lesions" of disseminated lupus ery-
ematous.

Laboratory studies: Urinalysis: albumin 1 to 4 plus, occasional erythrocytes, leucocytes
and granular casts in the sediment. Hemoglobin 6.0-13.8 Gm. per cent. Leukocytes 4350-
6700 per cu. mm. Differential count consistently normal. Total serum proteins: 5.5 Gm.
per cent, albumin 2.5 Gm. per cent, globulin 3.0 Gm. per cent, α globulin 1.0 Gm. per cent,
β globulin 0.8 Gm. per cent, γ globulin 1.2 Gm. per cent (Method of Milne). Blood urea
nitrogen 10 mg. per cent. Serum bilirubin: prompt direct 0.1 mg. per cent, total 0.3 mg.
per cent. Cephalin cholesterol flocculation 4 plus. Thymol turbidity 7 units. Zinc turbidity
12 units. Serum cholinesterase 1.36 A pH/hour. Bromsulphalein retention: 2 per cent in 45
minutes. Urine urobilinogen 1.4 mg./day. Heterophil antibody titer 1/7. Cold agglutinin
titer 1/7. Coombs test negative direct and indirect. Blood serology tests: Wassermann-
Kolmer 4 plus, Kahn 3 plus, quantitative Kahn 40 units, Kline 1 plus, Hinton negative,
VDRL weakly positive. Cerebrospinal fluid serology tests: Wassermann-Kolmer negative,
VDRL negative. Bone marrow examination showed a slight depression of normobla-
stic activity. No typical L.E. cells were encountered, although several heavier staining inclu-
sions were present in some neutrophils. Attempts to demonstrate L.E. cells in the buffy
coop of the patient's plasma or clotted blood and to induce the formation of these cells in
normal marrow mixed with the patient's serum failed repeatedly.

The majority of the tests of hemoastosis were abnormal: Bleeding, clotting, recalcification
and prothrombin time were prolonged and the Rumpel-Leede test was positive. The results

<table>
<thead>
<tr>
<th>Table 1.—Tests of hemoastosis</th>
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<tbody>
<tr>
<td>Test</td>
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<tr>
<td>---------------------------</td>
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<tr>
<td>Bleeding time—minutes</td>
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<tr>
<td>Clotting time—minutes</td>
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<tr>
<td>Prothrombin time—seconds</td>
</tr>
<tr>
<td>Recalcification time—seconds</td>
</tr>
<tr>
<td>Platelets—per cu. mm.</td>
</tr>
<tr>
<td>Clot retraction</td>
</tr>
<tr>
<td>Rumpel-Leede</td>
</tr>
<tr>
<td>Fibrinogen—mgm. %</td>
</tr>
</tbody>
</table>
| Prothrombin concentra-
   tion—units per ml.       | 54-69         | 165           | 186           | 250-380        |

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Table 2.—Effect of normal plasma on the abnormal coagulation tests in case 1

<table>
<thead>
<tr>
<th>Patient's blood</th>
<th>Patient's plasma</th>
<th>Normal plasma</th>
<th>0.9% NaCl Clotting time</th>
<th>Prothrombin time—seconds</th>
<th>Recalcification time—seconds</th>
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<tr>
<td>1.0</td>
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<td>0.1</td>
<td>64</td>
<td>66</td>
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<tr>
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<td>0.1</td>
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<td>67.0</td>
<td>870</td>
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Table 3.—One-stage prothrombin times of various plasma mixtures

<table>
<thead>
<tr>
<th>Plasma of case 1—ml.</th>
<th>Normal plasma—ml.</th>
<th>Labile factor deficient plasma—ml.</th>
<th>Stable factor deficient plasma—ml.</th>
<th>Dicumarol plasma—ml.</th>
<th>0.9% NaCl ml.</th>
<th>Prothrombin time—seconds</th>
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<tr>
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<td>0.1</td>
<td>69.6</td>
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<td>64.6</td>
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<td>14.6</td>
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<td>0.1</td>
<td>0.1</td>
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<td>0.1</td>
<td>0.1</td>
<td>13.6</td>
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<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>52.8</td>
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<td>0.1</td>
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<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>14.8</td>
</tr>
</tbody>
</table>

of these tests varied appreciably over a period of four years (table 6) and the figures presented in table 1 represent the maximal range of fluctuation. Very early in the course of this study it was noticed that the addition of normal plasma had no effect on the abnormal tests (table 2). This observation led to the suspicion of a circulating anticoagulant.

Demonstration of circulating anticoagulant.

The failure of normal plasma to affect the patient’s prothrombin time (table 3) strongly suggested that the very prolonged value of the patient’s own plasma was mainly caused by a clotting inhibitor and not by a deficiency of prothrombin or any of its conversion factors. Control studies presented in the same table demonstrate in fact that the addition of 0.1 ml. of normal plasma had a nearly corrective effect on the prolonged prothrombin time of 0.1 ml. of plasma deficient in stable factor, labile factor or prothrombin. It was also shown that the mixture 0.1 ml. of 0.9 per cent NaCl solution prolongs the prothrombin time of 0.1 ml. normal plasma only of 1.8 seconds. The value of 64.6 seconds obtained with a mixture of equal amounts of normal and patient plasma was evidently the result of an inhibitory effect of the patient’s plasma on the prothrombin time of normal plasma. The anticoagulant also influenced the clotting time of normal blood and recalcification time of normal plasma (tables 4 and 5). The lowest dilution in which the inhibitory effect could be demonstrated was 1:100. Anti Hemophilie Globulin (AHG) deficient plasma which had a prolonged recalcifi-
TABLE 4.—Effect of plasma of case 1 on clotting time of normal blood

<table>
<thead>
<tr>
<th></th>
<th>Plasma of case 1—ml.</th>
<th>Normal blood—ml.</th>
<th>Clotting time—minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.0</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>1.0</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>0.075</td>
<td>1.0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.0</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>1.0</td>
<td>35</td>
</tr>
<tr>
<td>AHG deficient plasma—ml.</td>
<td>0</td>
<td>0.05</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.0</td>
<td>1.0</td>
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<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Normal blood—ml.</td>
<td>0.05</td>
<td>1.0</td>
<td>15</td>
</tr>
<tr>
<td>Clotting time—minutes</td>
<td>17</td>
<td>16</td>
<td>15</td>
</tr>
</tbody>
</table>

TABLE 5.—Effect of abnormal plasmas on recalcification time of normal plasma

| Plasma of case 1—% | 100 80 60 40 20 10 5 2.5 1.0 0.5 0 | 100 |
| Normal plasma—%    | 0 20 40 60 80 90 95 97.5 99 99.5 100 |
| Recalcification time—seconds | 640 620 620 630 610 540 600 330 210 140 145 |
| Plasma of 3rd infant of case 1—% | 100 60 20 | 0 |
| Normal plasma—%    | 0 40 80 | 100 |
| Recalcification time—seconds | 720 700 605 |
| Plasma of case 2—% | 100 80 60 40 20 10 | 0 |
| Normal plasma—%    | 0 20 40 60 80 90 | 100 |
| Recalcification time—seconds | 480 465 450 450 360 330 150 |
| Plasma of case 3—% | 100 80 60 40 20 10 | 0 |
| Normal plasma—%    | 0 20 40 60 80 90 | 100 |
| Recalcification time—seconds | 480 490 480 460 460 380 |
| AHG deficient plasma—% | 100 80 60 40 20 10 5 | 0 |
| Normal plasma—%    | 0 20 40 60 80 90 95 | 100 |
| Recalcification time—seconds | 630 160 160 165 155 150 155 |

Recalcification time in the range of the patient's plasma was used as control: it had no inhibitory effect.

The concentration of prothrombin determined with the modified two-stage method ranged between 54 and 69 units per ml. of plasma. The short slope of the curve (fig. 1) obtained with this method indicates that there was no deficiency of prothrombin conversion factors. This was verified by the fact that fresh normal BaSO₄ treated plasma and normal 48-hour-old serum added as sources of labile and stable prothrombin conversion factor respectively, had no effect on the curve. The one-stage prothrombin time was not influenced either by these two sources of conversion factors. While the results obtained with the two-stage method were remarkably constant during the course of the patient's illness, the one-stage prothrombin times showed a fluctuation from 19.8 to 74.0 seconds (table 6). This discrepancy is most plausibly explained by a fluctuation
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Fig. 1. Two-stage prothrombin determination in plasma

Table 6.—Variations of abnormal tests in case 1 over a four year period

<table>
<thead>
<tr>
<th>Date</th>
<th>4-23-50</th>
<th>5-29-50</th>
<th>7-11-51</th>
<th>11-26-51</th>
<th>3-29-52</th>
<th>12-16-52</th>
<th>3-31-53</th>
<th>6-8-54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting time—minutes</td>
<td>101</td>
<td>50</td>
<td>56</td>
<td>51</td>
<td>50</td>
<td>57</td>
<td>60</td>
<td>42</td>
</tr>
<tr>
<td>Prothrombin time—seconds</td>
<td>20.1</td>
<td>20.5</td>
<td>20.9</td>
<td>19.8</td>
<td>63.0</td>
<td>74.0</td>
<td>29.2</td>
<td>26.0</td>
</tr>
<tr>
<td>Prothrombin concentration—units per ml</td>
<td>55</td>
<td></td>
<td>54</td>
<td>69</td>
<td>57</td>
<td>69</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>Recalcification time—seconds</td>
<td>640</td>
<td>540</td>
<td>810</td>
<td>1380</td>
<td>870</td>
<td>270</td>
<td>630</td>
<td></td>
</tr>
</tbody>
</table>

in inhibitor activity and the difference in plasma dilutions applied in running the two tests. The final dilution of the tested plasma is 1:300 for the two-stage method and 3:10 for the one-stage method. It is probable that the clotting inhibitor was diluted out to the point of ineffectiveness in the two-stage method, while the moderate dilution applied in running the one-stage test did not appreciably influence its activity. Further supportive evidence for this explanation was presented above (table 5) when it was demonstrated that the anticoagulant was effective up to, but not beyond, a dilution of 1:100. The fluctuation encountered with the one-stage method over a period of four years probably reflects changes in inhibitor activity. This fluctuation was reflected to a certain degree in the recalcification times, but not in the clotting times of venous blood. The last test is notoriously the least sensitive test of hemostasis and the most
subject to technical errors, hence it would be hazardous to draw any direct conclusions from the clotting times in Table 6. One can only state that the clotting times were consistently prolonged.

The consumption of prothrombin tested with the two-stage method was practically nil (Fig. 2). During spontaneous coagulation in glass tubes at 37°C over a period of two hours the concentration of prothrombin fell from 69 to 58 units per ml. To our surprise the one-stage method showed a progressive shortening of the serum prothrombin times as coagulation progressed, suggesting a rise in activity of prothrombin or its conversion factors. This phenomenon remains unexplained. The addition of 1:10 volume normal plasma had no effect on the rate of prothrombin consumption. The small amount of prothrombin present in normal plasma accounts for the slightly higher values of prothrombin concentration encountered with both methods when compared with the values obtained when the patient’s blood was tested alone. Control studies with Anti Hemophilic Globulin (AHG), Plasma Thromboplastin Component (PTC), and Plasma Thromboplastin Antecedent (PTA) deficient bloods with a prothrombin consumption in the range of the one observed with this patient’s blood, have shown that the addition of 1:10 volume normal plasma has a complete corrective effect. The patient’s consumption of prothrombin did not fluctuate during the four year period of observation, it remained minimal throughout. An interesting observation was the effect of the patient’s plasma on the consumption of prothrombin of normal blood (Fig. 3). The addition of 1:10 volume patient’s plasma had a definite inhibitory effect on the rate of prothrombin consumption of normal blood. This is in striking contrast with AHG, PTC and PTA deficient
Site of action of the anticoagulant.

The tests presented thus far indicate that the anticoagulant decreased the prothrombin consumption and prolonged the clotting time, prothrombin time, and recalcification time of the patient's blood. The inhibitor was also capable of inducing the same abnormalities in normal blood. The hypoprothrombinemia encountered with the two-stage method accounts in minor part for the prolongation of the patient's one-stage prothrombin time, but it is very unlikely that it had any effect on clotting and recalcification time. Control studies with blood from patients treated with Dicumarol in which the prothrombin concentration was approximately 50 U/ml. have shown that their clotting and recalcification times were only moderately, if at all, elevated, and this in spite of the presence of a complicating stable factor deficiency induced by Dicumarol. From a theoretical standpoint this group of abnormalities could be caused by heparin or by an inhibitor of thromboplastin or of the labile factor of prothrombin conversion. An anticoagulant of the heparin type was excluded by normal thrombin clotting times (table 7) and by the failure of various concentrations of protamine sulfate to affect the patient's prothrombin and recalcification times (table 8).

The presence of a inhibitor of the labile factor is highly questionable, because if one makes the assumption that the prothrombin time of 19.8 seconds observed on November 26, 1951 was caused by a combined hypoprothrombinemia and...
labile factor inhibition, it is scarcely conceivable that such a weak inhibitor could account for a clotting time of 51 minutes and a consumption of prothrombin which was practically nil. We have recently had the opportunity to study a case of labile factor deficiency with a prothrombin time of 29.8 seconds and Alexander reported three well documented similar cases with prothrombin times over 19.8 seconds whose clotting time, recalcification time and prothrombin consumption were by far not as abnormal as in the case presented here.

The only inhibitor which would explain all the findings is an antithromboplastin. The results of the test devised to demonstrate an antithromboplastin (fig. 4) have to be interpreted with some reserve. First, one should realize that the times obtained with normal plasma are not directly comparable with the times obtained with the patient’s plasma because the starting values with undiluted thromboplastin are different. A more comparable control plasma was Dicumarol plasma with a prothrombin time of 20.2 seconds. It appears quite obvious that in low dilutions of thromboplastin the values obtained with the patient’s plasma are much longer than with the control plasmas. This is presumptive but not conclusive evidence for an anti-thromboplastin. It has been shown by Langdell et al. that AHG and PTC deficient plasmas give abnormal results similar to those encountered in our patient. It was therefore necessary to exclude an inhibitor of AHG and PTC. The patient’s plasma was tested on AHG and PTC deficient blood. It had no effect on the abnormal clotting time and prothrombin consumption of any of them. This was not surprising, and actually expected in view of the results presented in table 4 and figure 3 where the patient’s plasma was shown to inhibit the same tests in normal blood. The patient’s plasma was also ineffective on PTA deficient and thrombocytopenic blood; on the contrary it made the abnormalities more pronounced in both conditions. The inability of demonstrating AHG, PTC and PTA activity in the patient’s plasma does not imply that the inhibitor acted against these three factors. AHG, PTC
and PTA activity can only be demonstrated by determining their ability to form thromboplastin which induces prothrombin conversion to thrombin which in turn finally coagulates fibrinogen. An anticoagulant which blocks thromboplastin activity precludes the testing of non-formed plasma thromboplastin precursors. In the present case it is highly unlikely that the inhibitor had a multiplicative effect on all three precursors, or that there were actually three different inhibitors each of them acting against one of the three plasma factors. A more convincing and absolute argument against an inhibitor of thromboplastin precursors is the retarding effect of the anticoagulant on the prothrombin time. This cannot be explained by a disturbance in the phase of thromboplastin formation; it is well known in fact that AHG, PTC and PTA deficiency, no matter how severe they are, all have a normal prothrombin time. The same argument excludes an inhibitor of platelet activity. The elimination of an AHG, PTC, PTA or platelet inhibitor leaves only one type of anticoagulant to account for the results presented in figure 4: an antithromboplastin. For the sake of completeness one should also consider an inhibitor of prothrombin or of the stable factor of prothrombin conversion. This can be excluded on theoretical grounds by the marked delay in prothrombin consumption observed in our patient. Hypoprothrombinemic and stable factor deficient bloods have normal prothrombin consumption when tested over a period of 2 hours. Furthermore, it was shown that the patient had a prothrombin time of 19.8 seconds on November 26, 1951. Assuming that this was due to an inhibitor of prothrombin or stable factor, such a weak inhibitor could not account for the marked prolongation of clotting time and recalcification time. Blood from patients treated with Dicumarol or from
patients with congenital stable factor deficiency with a prothrombin time above 19.8 seconds have only a moderate prolongation of the clotting and recalcification time.

The rather pronounced prolongation of the bleeding time deserves a special comment in the light of the conclusion drawn above. Hemostasis following a lancet stab applied for determination of the bleeding time is afforded by a platelet thrombus and vasoconstriction. Platelet agglutination depends upon prompt availability of thrombin. In the present case the formation of thrombin was markedly delayed by the anticoagulant which appeared to inhibit not only plasma thromboplastin, but tissue thromboplastin as well. All tests reported herein which involved the use of thromboplastin were performed with tissue thromboplastin prepared from acetone dried rabbit brain. It is believed therefore that the inhibitor accounted, at least in part, for the prolongation of the bleeding time. It cannot be excluded however, that increased capillary fragility, demonstrated by the positive Rumpel-Leede test, may have played a contributory role.

The anticoagulant was not dialyzable. It resisted heating at 60 C. for 30 minutes, was partially inactivated at 70 C. for 10 minutes and completely destroyed at 70 C. for 30 minutes. BaSO₄ did not adsorb the inhibitor from oxalated plasma.

Demonstration of the anticoagulant in the patient’s third child.

The study of this baby girl revealed several interesting features. As seen in table 9 she had the same clotting abnormalities as her mother with the exception of the bleeding time which was normal. The anticoagulant could be demonstrated during the first seven weeks of life. At the end of seven weeks there still was a definite prolongation of the recalcification time and the prothrombin time was two seconds longer than the normal control plasma. At the end of three

<table>
<thead>
<tr>
<th>Test</th>
<th>Age of infant</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>three days</td>
</tr>
<tr>
<td>Clotting time—minutes.............</td>
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</tr>
<tr>
<td>Bleeding time—minutes.............</td>
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<tr>
<td>Plasma prothrombin—time-seconds</td>
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<td>Recalcification time—seconds</td>
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<td>Platelets—per cu. mm..............</td>
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<td>Clot retraction</td>
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<tr>
<td>Serum prothrombin time two hours after veni-puncture—seconds</td>
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</tr>
<tr>
<td>Blood</td>
<td>Wassermann-Kolmer</td>
</tr>
<tr>
<td>Kline</td>
<td>Negative</td>
</tr>
<tr>
<td>Kahn</td>
<td>—</td>
</tr>
<tr>
<td>Cephalin cholesterol flocculation</td>
<td>4+</td>
</tr>
</tbody>
</table>

TABLE 9.—Laboratory tests on the third infant of case 1
months all tests were normal. The infant also had abnormal serologic tests and a positive cephalin cholesterol flocculation which persisted for six months. The remaining laboratory tests were as follows: Hemoglobin 18.0 Gm. per cent. Urinalysis: Very occasional leukocytes in the sediment. Serum bilirubin: prompt direct 0.1 mg. per cent, total 0.5 mg. per cent. Thymol turbidity, 4 units. Zinc turbidity, 1 unit. Total serum proteins 5.4 Gm. per cent, albumin 3.4 Gm. per cent, globulin 2.0 Gm. per cent. The analogy with the maternal findings can only be explained by the transplacental transfer of the anticoagulant and the factor(s) which caused the serologic and turbidimetric abnormalities. The divergent behavior in respect to survival time in the baby’s circulation strongly suggests that the anticoagulant cannot be identified with the factor(s) accounting for the positive Wassermann and cephalin cholesterol flocculation. On the other hand, it is of interest that both anticoagulant and the plasma component causing the false positive serology had similar physico-chemical characteristics. None of them could be dialyzed, heat resistance was similar and it was not possible to adsorb them with BaSO4. The child never had any hemorrhagic symptoms and developed normally.

Case 2. This 57 year old white male was hospitalized twice because of symptoms of arterial insufficiency in his lower extremities. He underwent a right lumbar sympathectomy in February 1953 without any hemorrhagic complications. In October 1953 he was re-admitted for a sympathectomy on the left side and an endarterectomy of the right common iliac artery. Shortly before the second hospital admission, the patient developed an itching skin rash secondary to aureomycin and penicillin therapy for a pyelitis. The patient did not have any unusual blood loss at the time of surgery on October 5, 1953. He was started prophylactically on penicillin and streptomycin on the day of surgery, but these drugs had to be discontinued on the sixth postoperative day because of recurrence of the itching rash which was accompanied by blood eosinophilia. The preoperative bleeding time was 2 minutes 45 seconds, the clotting time (capillary method) was 5 minutes 50 seconds. A clotting time done 24 hours after surgery as reference for future heparin therapy was reported as “longer than 7 minutes.” A Lee White clotting time done on the same day was 21 minutes (normal range up to 20 minutes). Heparin therapy was deferred and three days after surgery the clotting time was 29 minutes. There was a progressive spontaneous rise up to 82 minutes on the 8th postoperative day. Two weeks after surgery the clotting time was 50 minutes. To the surgeons surprise and delight there was only a small wound hematoma and the operative result was excellent. Unfortunately, it has not been possible to follow the patient after discharge from the hospital because his residence is more than 500 miles away. In a recent letter he did not mention any episodes of bleeding. There was no past personal or family history of hemorrhagic diathesis.

Laboratory data preoperatively (before October 5, 1953): Hemoglobin 15.0 Gm. per cent. Leukocytes 5500 per cu.mm. Differential count normal except for 6 to 9 per cent eosinophils. Urinalysis: Specific gravity 1.016, albumin trace, occasional leukocytes and erythrocytes in the sediment. Mazzini: Negative. Laboratory data postoperatively (between October 6 and 20, 1953): Mazzini 2 plus, Wassermann-Kolmer 4 plus, Kline negative, Kahn 2 plus, VDRL weakly positive. Cephalin cholesterol flocculation 4 plus. Total serum proteins 7.1 Gm. per cent, albumin 2.8 Gm. per cent, globulin 4.3 Gm. per cent, α globulin 1.4 Gm. per cent, β globulin 1.1 Gm. per cent, γ globulin 1.8 Gm. per cent. Coombs test: Negative direct and indirect. Routine tests of hemostasis are presented in table 1. Prothrombin consumption was minimal; as in case 1 it was noted that the one-stage values became progressively shorter as blood clotted spontaneously in glass tubes. While the plasma prothrombin time was 17.4 seconds, the serum prothrombin time was 13.8 seconds 3 hours after venipuncture. The thrombin titration was normal. The patient’s plasma prolonged the recalcification time of normal plasma (table 5). Even though time limitations did not
permit as extensive a study as in case 1, the identity of the results obtained strongly sug-
ests that the clotting abnormality of the two cases was the same, i.e., it was caused by
an antithromboplastin and a mild hypoprothrombinemia. The anticoagulant was not
dialyzable, it was resistant to 65 C. for 30 minutes and progressively lost its activity when
heated to 70 C.

Case 5. This 33 year old housewife was referred to the University of Minnesota Hospitals
in November 1952 because of arthralgia and anemia of one month duration. Studies of
hemostasis were performed in December 1953. The physical examination revealed pallor
and hepatosplenomegaly. The patient had menorrhagia.

Laboratory tests: The urine contained no albumin. Hemoglobin 7.7 Gm. percent. Erythro-
cytes 2,330,000 per cu.mm. Leukocytes 3100 per cu.mm. with a normal differential count.
Reticulocytes 7.1 per cent. Fecal urobilinogen 2048 Ehrlich units per 100 Gm. of stool.
Coombs test negative direct and indirect. Cold agglutinin titer 1:112. Serum bilirubin:
prompt direct 0.2 mg. per cent, total 1.7 mg. per cent. Cephalin cholesterol flocculation 4
plus. Thymol turbidity 12 units. Zinc turbidity 15 units. Total serum proteins 7.4 Gm.
per cent, albumin 3.0 Gm. per cent, globulin 4.4 Gm. per cent, α globulin 1.3 Gm. per cent,
β globulin 1.2 Gm. per cent and γ globulin 1.9 Gm. per cent. Serologic tests on blood:
Kolmer 4 plus, Hinton 4 plus, Kahn 4 plus, VDRL positive in dilution 1:32. Cerebrospinal
fluid Wassermann negative. The patient's bone marrow showed typical L.E. cells and
normoblastic hyperplasia. The diagnosis of lupus erythematosus with associated hemolytic
anemia was hence established. The patient was transfused and given peroral Cortisone
for six months. When seen in December 1953, the patient felt much better. Hemolysis and
anemia had disappeared, and it was not possible at this time to demonstrate any L.E.
cells. The abnormal serologic tests and the positive cephalin cholesterol flocculation
persisted however. Tests of hemostasis are presented in table 1. Clotting time and pro-
thrombin time were slightly prolonged: the recalcification time was 480 seconds and there
was moderate hypoprothrombinemia by the two-stage method. Prothrombin consumption
was at the lower limit of normal. Thrombin titration was normal. The patient's plasma
inhibited the recalcification time of normal plasma as observed with the other two cases
(table 5). The inhibitor was not dialyzable and had the same heat resistance characteristics
found in case 1.

Discussion

The appearance of circulating anticoagulants in the various conditions conventio-
nally grouped under the term "collagen disease" adds a new facet to the
already diverse and metamorphous clinical and laboratory picture of this disease
group. The occurrence of clotting inhibitors in patients with manifestations of
hypersensitivity may have some bearing on their mechanism of development.
The tendency of patients with lupus erythematosus disseminatus to develop
antibodies is a well established fact: the false positive serology and positive
Coombs test are frequent manifestations of this disease. It appears highly probable
that the mechanism of development of the anticoagulant is immunologic
in type as well. The association with the above listed antigen-antibody reactions
and the transplacental transfer in case 1 tend to substantiate this assumption.
The analogy to the transplacental transfer of maternal immune antibodies against
diphtheria and measles is obvious. This mechanism implies that a patient pro-
duces antibodies against antigens of his own plasma. This is in conflict with
Ehrlich's dictum, but there is good evidence that the production of such anti-
bodies can occur: auto-immune hemolysins constitute the most typical example.
The antigen which induces antibody formation in collagen disease is usually
unknown. Only in the case of periarteritis nodosa are there some leads suggest-
ing that antibiotics may play an antigenic role. It appears quite possible that
in case 2 the production of the anticoagulant was set off by penicillin. Unfortunately, it has not been possible to follow the patient long enough to see if the anticoagulant disappeared. If this were the case it would have been very instructive to see whether small doses of the antibiotic would induce the formation of the inhibitor.

The mechanism of action of the anticoagulant is probably directed against thromboplastin. It is rather remarkable that its physico-chemical characteristics (heat resistance, non-dialyzability and failure to be adsorbed on BaSO₄) are shared by the factor(s) which cause(s) a false positive serology. As mentioned earlier, however, the inhibitor cannot be identified with the antibody(ies) causing the abnormal serologic tests because its survival time in the infant of case 1 was much shorter than that of the other antibody(ies). Blood from several individuals with a positive Wassermann reaction caused by a luetic infection did not reveal any anticoagulant or abnormal cephalin cholesterol flocculation.

The transplacental transfer of the anticoagulant is the second reported instance of this phenomenon. The first report was published by Frick in 1953. In the original case the transfer occurred in an otherwise normal mother with no clinical or laboratory evidence of “collagen disease.” The anticoagulant was not anti-thromboplastin, but inhibited one of the plasma precursors of thromboplastin. It appears quite clear that the two anticoagulants were not identical even though the mechanism of development was probably immunologic in type in both instances.

The triad of anticoagulant, positive cephalin cholesterol flocculation and false positive blood serology was a common feature of all three cases. This does not necessarily imply that these laboratory abnormalities do always occur in combination. Cases of “collagen disease” have been encountered during the course of this study where the false positive serology was an isolated finding or where it was accompanied by a positive cephalin cholesterol flocculation without evidence of anticoagulant. Vice versa, all patients with clotting inhibitor presented the complete triad.

From a clinical standpoint it is remarkable that cases 1 and 2 who were most severely afflicted did not have more symptoms. If one resorts to congenital hemorrhagic diatheses for analogy, it appears that the patients presented herein had fewer symptoms than individuals with Anti Hemophilic Globulin (AHG) or Plasma Thromboplastin Component (PTC) deficiency with comparable abnormal tests. They also had fewer hemorrhages than the previously reported case with an acquired circulating anticoagulant with transplacental transfer where the inhibitor was believed to act at the first phase of normal coagulation, i.e., the phase of thromboplastin formation. The hemorrhagic symptomatology of cases 1 and 2 is much more comparable with congenital deficiencies involving clotting factors influencing the second phase of coagulation, i.e., the conversion phase of prothrombin to thrombin. It is well known that thromboplastin, labile and stable thrombin conversion factors influence this phase. On the basis of laboratory tests an inhibitor of labile or stable factor was excluded, hence, these clinical data add further supportive evidence to the assumption that the inhibitor was an anti-thromboplastin.
Very little comment can be made in regard to therapy. In case 1 a course of Cortisone in small doses for two months had no effect on the anticoagulant. Considering the spontaneous fluctuation of inhibitor activity observed in this case, it would be rather difficult to assess the value of any therapeutic agent. It is possible however, that prolonged treatment with larger doses of Cortisone may influence the anticoagulant; the effect of this drug on other types of immune antibodies like hemagglutinins has been well established.

The incidence of anticoagulants in “collagen disease” appears rather low. A total of thirty patients including sixteen cases of lupus erythematosus disseminatus with positive L.E. cell phenomenon, nine cases of periarteritis nodosa, three cases with a systemic reaction to penicillin, and two unclassified cases with multiple manifestations of hypersensitivity were studied. The three cases where the presence of an anticoagulant could be demonstrated beyond doubt represent only 10 per cent of the total group: two had lupus erythematosus disseminatus, and one had an allergic reaction to penicillin. No case of histologically proven periarteritis nodosa revealed clotting inhibitors.

SUMMARY

1. A circulating anticoagulant was demonstrated in three patients with “collagen disease.” In all cases it was associated with a false positive blood serology and a four plus cephalin cholesterol flocculation.

2. In one instance, the anticoagulant was transferred to a newborn infant where it persisted for seven weeks. The infant also demonstrated the abnormal serologic and turbidimetric tests during the first six months of life.

3. The anticoagulant probably acted as anti-thromboplastin.

4. The occurrence of a clotting inhibitor in association with other manifestations of hypersensitivity and its transplacental transfer strongly suggest that the mechanism of development is immunologic in type.

5. This study included a total of thirty patients with lupus erythematosus disseminatus and associated conditions conventionally grouped under the term “collagen disease.” The incidence of circulating anticoagulants was 10 per cent.

SUMMARIO IN INTERLINGUA

1. Esseva demonstrate in 3 patientes con “morbo collagenic” le presentia de un anticoagulante circulante. In omne casos le anticoagulante circulante esseva associate con un serologia sanguinee falsemente positive e un flocculation cholesterolic a cephalina de grado 4 plus.

2. In un caso le anticoagulante se trovava, per transferimento transplacental, in le neonate infante del patiente. Illac illo persisteva durante un periodo de 7 septimanas.

3. Le anticoagulante ageva probabilmente como anti-thromboplastina.

4. Le occurrentia de un inhibitor de coagulamento in association con altere manifestationes de hypersensibilitate e le transferimento transplacental de ille inhibitor es un forte indice de que su mechanismo disveloppamental es de charac-ter immunologic.

5. Le studio hic presentate includeva un total de 30 patientes con disseminate
CIRCULATING ANTICOAGULANTS IN "COLLAGEN DISEASE"

lupus erythematoso e un associate condition conventionmente classificate como "morbo collagenic." Le frequentia de anticoagulantes circulante esseva 10 pro cento.

REFERENCES


Acquired Circulating Anticoagulants in Systemic "Collagen Disease": Auto-immune Thromboplastin Deficiency

PAUL G. FRICK and MARY K. WEIMER