A FACTOR IN SERUM WHICH ACCELERATES THE CONVERSION OF PROTHROMBIN TO THROMBIN

III. Its Relationship to the Coagulation Defect of Thrombocytopenic Blood

By Benjamin Alexander, M.D., and Andre de Vries, M.D.

With the technical assistance of Eunice Addelson

The exact role of the platelet in blood coagulation is the subject of considerable controversy. Although thrombocytopenic plasma exhibits retarded coagulation, a prolonged clotting time is rare in thrombocytopenic purpura. This has been explained by the theory that even in severe thrombocytopenia sufficient thromboplastin is elaborated to produce normal coagulation. In any event, the hemorrhagic manifestations of thrombocytopenic purpura have generally been ascribed either to a great reduction in blood platelets, to capillary dysfunction or to inadequate clot retraction rather than to abnormalities in coagulation itself.

It is the purpose of this paper to present observations which indicate that the coagulation of thrombocytopenic blood is profoundly disturbed.

In a previous communication an agent was described in serum which accelerates the conversion of prothrombin to thrombin in the presence of thromboplastin plus calcium. While insufficient data are available to establish the identity or non-identity of this substance with other factors reported to have similar attributes, some of its biochemical and physiologic properties have been described, a method for its determination given, and its elaboration in the coagulation of normal blood delineated.

METHODS

The agent, serum prothrombin conversion accelerator (SPCA), is measured by the enhancement, in percent, of the prothrombin activity of normal oxalated plasma induced by the admixture to it of serum obtained from the blood in question one hour after coagulation. Before the test, the serum is oxalated, and incubated for one-half hour in order to inactivate thrombin.

The prothrombin activities of plasma and serum were determined by modifications of the one stage procedure; coagulation time was measured by a modification of the Lee and White technic. Platelets were enumerated by the method of Rees and Ecker, and bleeding time was determined by the Duke method.

RESULTS

Ten subjects† with thrombocytopenic purpura were studied (table 1). All had platelet counts below 100,000 per mm. The mean SPCA activity was 33 percent in contrast to 99 for 95 normal subjects previously reported. The residual serum

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prothrombin activity averaged 50 per cent* compared with 6 per cent for normal individuals. The algebraic differences between plasma and serum prothrombin activities averaged 37.5, whereas in normals they average 90+.

**Table 1.** — Prothrombin Consumption and Evolution of Serum Prothrombin Conversion Accelerator in Coagulation of Thrombocytopenic Blood

<table>
<thead>
<tr>
<th>Subj.</th>
<th>Disease</th>
<th>Plate. no.</th>
<th>Bleed. time</th>
<th>Clot. time</th>
<th>Prothrom. activity,* per cent</th>
<th>SPCA per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. P.</td>
<td>Idiopathic thromb. purpura</td>
<td>13</td>
<td>4</td>
<td>13</td>
<td>97</td>
<td>47</td>
</tr>
<tr>
<td>H. J.</td>
<td>Idiopathic thromb. purpura</td>
<td>22</td>
<td>&gt;10</td>
<td>2</td>
<td>100</td>
<td>55</td>
</tr>
<tr>
<td>D.</td>
<td>Idiopathic thromb. purpura</td>
<td>11</td>
<td>14</td>
<td>11</td>
<td>70</td>
<td>19</td>
</tr>
<tr>
<td>M.</td>
<td>Idiopathic thromb. purpura</td>
<td>97</td>
<td>—</td>
<td>11</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>C. G.</td>
<td>Idiopathic thromb. purpura</td>
<td>70</td>
<td>4</td>
<td>9</td>
<td>141</td>
<td>127</td>
</tr>
<tr>
<td>M. S.</td>
<td>Idiopathic thromb. purpura</td>
<td>30</td>
<td>3</td>
<td>10</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>B. M.</td>
<td>Cirrhosis, splenomegaly</td>
<td>69</td>
<td>5</td>
<td>14</td>
<td>96</td>
<td>25</td>
</tr>
<tr>
<td>S. S.</td>
<td>Hodgkin's dis. Nitrogen mustard therapy</td>
<td>76</td>
<td>6</td>
<td>11</td>
<td>60</td>
<td>67</td>
</tr>
<tr>
<td>W.</td>
<td>Gaucher's disease</td>
<td>67</td>
<td>—</td>
<td>16</td>
<td>65</td>
<td>58</td>
</tr>
<tr>
<td>X.</td>
<td>Multiple myeloma</td>
<td>94</td>
<td>—</td>
<td>—</td>
<td>58</td>
<td>47</td>
</tr>
</tbody>
</table>

* All values corrected for dilution with oxalate.

**Table 2.** — Effect of Normal Plasma, Platelets, or Thromboplastin on Coagulation of Thrombocytopenic Blood

<table>
<thead>
<tr>
<th>Patient M. S. Idiopathic Thrombocytopenic Purpura</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Platelet</th>
<th>Clotting time</th>
<th>SPCA</th>
<th>Serum proth. activity</th>
<th>Plasma minus serum proth. activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood alone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 cc. blood plus 0.1 cc. normal plasma</td>
<td>60</td>
<td>14</td>
<td>28</td>
<td>67</td>
</tr>
<tr>
<td>2 cc. blood plus 0.2 cc. normal plasma</td>
<td>74</td>
<td>15</td>
<td>34</td>
<td>52</td>
</tr>
<tr>
<td>2 cc. blood plus platelets from 0.2 cc. normal plasma*</td>
<td>87</td>
<td>15</td>
<td>47</td>
<td>44</td>
</tr>
</tbody>
</table>

Patient C. G. — Idiopathic Thrombocytopenic Purpura

| Blood alone | 70 | 9 | 18 | 115 | 3 |
| 2 cc. blood plus 0.1 cc. thromboplastin sol.† | 70 | <1 | 165 | 17 | 101 |

* 1.0 cc. oxalated plasma was centrifuged at 3000 r.p.m. for 30 minutes. The supernatant plasma was decanted and the sediment suspended by stirring and vigorous shaking in 1.0 cc. of physiologic saline. 0.1 cc. of the mixture was added to 2 cc. of the patient's blood.

† Thromboplastin solution prepared from Difco commercial thromboplastin as for prothrombin determination.

No strict correlation was evident between the bleeding time or platelet count on the one hand and the SPCA or residual serum prothrombin activity on the other,

* Normal plasma is considered to have 100 per cent prothrombin activity.
although those subjects with the highest platelet counts seemed to have the highest SPCA activities. The coagulation times of most of the patients were within the accepted range of normality.

The addition of normal oxalated plasma, platelets or thromboplastin extract to shed thrombocytopenic blood accelerated coagulation, increased prothrombin consumption, and increased the amount of SPCA evolved (table 2).

Of considerable interest are the observations in one subject with idiopathic thrombocytopenic purpura before and after splenectomy (table 3). Despite the fact that the platelet count and the bleeding time returned to normal following the procedure, there was practically no change in the SPCA. Residual serum prothrombin did, however, decrease somewhat, but as the patient relapsed about one month after operation, it again increased.

**DISCUSSION**

Evolution of SPCA during coagulation is enhanced by supplements of thromboplastin to, or by mechanical agitation of, clotting blood. Conversely, it is markedly reduced by inhibiting coagulation by exposing blood to siliconized surfaces, a condition which interferes with thromboplastin elaboration. Concomitantly, residual serum prothrombin activity is greatly increased.

The similar observations in thrombocytopenia indicate that a decreased number of platelets is associated with insufficient evolution of thromboplastin. This results in abnormally small prothrombin conversion to thrombin associated with inadequate SPCA evolution, as a consequence of which the conversion of additional prothrombin to thrombin is retarded. That the clotting times were essentially.
normal despite the clotting defect reflects the lack of sensitivity of this test. Similarly in dicumarolized plasma the coagulation time is, more often than not, normal while SPCA is small.7 And in hemophilia12 comparable abnormalities in residual prothrombin activity and SPCA elaboration are observed even when the clotting time is restored toward normal by the addition or normal plasma or thromboplastin extracts. These observations are understandable when it is realized that in the coagulation of blood only a very small fraction of the total plasma prothrombin need be converted to thrombin to give a normal clotting time.14

From the experiments on one subject before and after splenectomy it appears that restoration of the platelet count to normal did little to remedy the clotting defect. What relation this had to the prompt relapse of the thrombocytopenia with clinical manifestations of bleeding is obscure and demands further exploration. It seems that although the patient had a normal number of circulating platelets following operation, they may not have been qualitatively satisfactory for rectifying the defect in coagulation. This is substantiated by the fact that the addition of normal plasma or platelets therefrom to the blood of this same subject corrected the abnormality. Such a concept is in accord with interpretations by Aggeler et al. of evidence regarding variability in the functional capacity of platelets.15

The significance of these abnormalities in the pathogenesis of the hemorrhagic phenomena of thrombocytopenic purpura requires further investigation. According to Allen et al.16 a circulating heparin-like anticoagulant may be present in idiopathic thrombocytopenic purpura. The clotting defect observed by us in this disease cannot be attributed to heparin since we found7 that the addition of moderate amounts of heparin to freshly drawn normal blood so as to retard coagulation substantially failed to inhibit SPCA evolution or prothrombin conversion to thrombin.

**Summary**

The sera from thrombocytopenic blood show abnormally large residual prothrombin activity and small amounts of prothrombin conversion accelerator. The addition of normal platelets or thromboplastin corrects these abnormalities. In one subject the clotting defect persisted despite temporary remission of the thrombocytopenia consequent to splenectomy.

**References**

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