STUDIES ON BLOOD COAGULATION

IV. THE NATURE OF THE CLOTTING DEFICIENCY IN HEMOPHILIA

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Numerous explanations, recently summarized by Wöhlsch, have been offered for the delayed coagulation observed in hemophilia, the presumable cause of the bleeding tendency. According to Sahli, Nolf, Morawitz, Minot and Lee, Howell and Cekada, and Christie, the platelets of hemophilic blood are excessively stable, failing to disintegrate and yield the platelet factor essential for coagulation. However, Addis, Klinger, and more recently, Feissly and Fried maintain that the platelets are functionally normal. The latter find hemophilic blood to be deficient in prothrombin, this deficiency presumably explaining the retarded coagulation. Addis, however, reports a qualitative difference in so far as the prothrombin of hemophilic blood, although normal in quantity, is more slowly activated by Ca and the cell factor to form thrombin. These findings are disputed by Howell and Cekada.

Clearly, all these theories cannot be correct; and the experiments to be described seem to confirm the observations of Addis. The platelets of normal and hemophilic\(^1\) blood compared quantitatively with respect to their effect upon the coagulation of platelet-free horse or human plasma, are found to be indistinguishable. Both types of platelet suspension accelerate coagulation to the same degree, and both accelerate the production of thrombin from prothrombin to the same degree, no matter what type of plasma is used, whether oxalated,

\(^1\) Dr. W. B. Chew of the Boston City Hospital, Thorndyke Laboratory, kindly furnished the blood from hemophilic patients under treatment or observation for use in these experiments.
citrated, horse, rabbit, normal human, or hemophilic. Confirming the
findings of Christie and coworkers and of Addis there is no difference
in the prothrombin contents of normal and hemophilic plasma, both
ultimately developing the same quantity of thrombin upon the addi-
tion of platelets and calcium. Finally, normal and hemophilic plasma
are equally coagulable by thrombin, indicating that they do not differ
in their antithrombin activity, as claimed by Fuchs.

The one fundamental difference between hemophilic and normal
plasma, apparent in all the following experiments, and illustrated in
Fig. 1, is the fact that the rate of thrombin production in hemophilic
plasma or in solutions of prothrombin derived from such plasma is
very much less than in normal plasma, an adequate explanation of the
delayed coagulation time and prolonged bleeding. As might be
expected, therefore, if an excess of platelets, whether normal, hemo-
philic, or animal in origin, is added to hemophilic plasma prior to
recalcification, it coagulates normally, and if a large excess of platelets
is used, hemophilic plasma can be made to coagulate even more rapidly
than normal plasma.

The cause of the delayed activation of the prothrombin of hemo-
philic plasma, despite the fact that it is present in normal concentra-
tion and that the platelets function normally, is still speculative. The
prothrombin may be deficient in an essential activating factor, or, as
suggested by Fuchs, there may be an inhibiting factor analogous to
heparin.

Methods and Materials

Prothrombin, thrombin, fibrinogen, and platelet suspension were
prepared and tested as described in a preceding paper (Eagle).

1. The Antithrombin Activity of Hemophilic Plasma Is Normal.—

| Horse thrombin added to 1 cc. plasma, cc. | 0.4 | 0.2 | 0.1 | 0.05 | 0.025 |
| Coagulation time, normal human plasma, min. | 5   | 10  | 60  | 4 hours |
| Coagulation time, hemophilic plasma, min. | 4½  | 8   | 50  | Greater than 4 hours |

Qualitatively the same results were obtained when horse or human
thrombin was added to normal and hemophilic serum, the mixture
incubated for \( \frac{1}{2} \) hour, and its thrombin activity then tested by adding serial quantities to 1 cc. of pure horse fibrinogen solution. There was no demonstrable difference between any of the four hemophilic plasmas and five normal human controls.

2. **The Coagulating Activity of Hemophilic Platelets Is Normal.**—Serially decreasing quantities of similar washed suspensions of normal and hemophilic platelets were added to 1 cc. of citrated (a) normal human plasma, (b) hemophilic plasma, (c) horse plasma, and (d) horse prothrombin solution; CaCl\(_2\) was then added and the coagulation time noted. The human plasmas had been freed of platelets by centrifugation, the horse plasma by centrifugation and Berkefeld filtration. The amount of 0.1 m CaCl\(_2\) used was usually 1/5 the plasma volume for human citrated plasma (0.5 per cent), and 1/2 the plasma volume for horse plasma. With oxalated plasmas, four times the amount necessary to combine with the oxalate stoichiometrically was used. In each experiment, the total value was always brought to two times the volume of plasma by adding 0.85 per cent NaCl. In no case was there any significant difference in the coagulating activity of normal and hemophilic platelets (Table I).

3. **The Prothrombin Content of Hemophilic Plasma Is Normal.**—
Fibrinogen-free solutions of prothrombin were prepared from normal and hemophilic plasma by the method described in a preceding paper (Eagle). After the addition of 1/20 volume of 0.1 m CaCl₂, the thrombin content in each solution was measured at intervals. As is shown in Fig. 1, the amount of thrombin ultimately formed in the two solutions is the same, within the normal limits of variation.

4. Hemophilic Plasma Differs from Normal Plasma in the Slow Liberation of Thrombin upon the Addition of Calcium.—The basic reason for the delayed coagulation of hemophilic plasma is indicated by the fact that if serially increasing quantities of a platelet suspension are added to platelet-free normal and platelet-free hemophilic plasma, the acceleration of coagulation with each increment of platelets is much greater with the former than it is with hemophilic plasma; but, if a sufficiently large excess of platelets is added to the latter, it can be made to coagulate in less than the normal coagulation time (Table II). Since, as has been shown in a preceding paper, the only known effect of the platelets is to accelerate the rate of thrombin formation, it seems likely that the deficiency of hemophilic plasma consists of a delayed liberation of thrombin. This is confirmed by the following experiment.

**TABLE II**

*Showing That Hemophilic Plasma Can Be Made to Clot Normally by Using an Excess of Platelets*

Serially increasing quantities of horse platelets were added to 1 cc. of platelet-free normal and hemophilic plasma, the volume adjusted to 1.5 cc., and 0.5 cc. of 0.1 m CaCl₂ added to all the tubes.

<table>
<thead>
<tr>
<th>Concentrated suspension of horse platelets, cc.</th>
<th>0.2</th>
<th>0.05</th>
<th>0.0125</th>
<th>0.0031</th>
<th>0.0008</th>
<th>0.0002</th>
<th>0.00005</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation time of normal plasma, min.</td>
<td>5½</td>
<td>6½</td>
<td>8</td>
<td>10</td>
<td>15</td>
<td>19</td>
<td>40</td>
<td>180</td>
</tr>
<tr>
<td>Coagulation time of hemophilic plasma, min.</td>
<td>6½</td>
<td>10</td>
<td>19</td>
<td>27</td>
<td>48</td>
<td>85</td>
<td>115</td>
<td>&gt;180</td>
</tr>
</tbody>
</table>

* Original coagulation time of the normal plasma, before removal of the platelets by Berkefeld filtration, was 9 minutes; the original coagulation time of the hemophilic plasma, before removal of the platelets by prolonged centrifugation, was 135 minutes.
Prothrombin was prepared from platelet-free normal and platelet-free hemophilic plasma. To 1 cc. of each prothrombin solution was added 0.1 cc. of CaCl₂ and the course of thrombin production followed quantitatively (cf. Eagle). As is shown in Fig. 1, the hemophilic prothrombin is converted to thrombin much more slowly than is normal prothrombin, but the amount of thrombin ultimately formed is normal. Since the latter depends solely upon the prothrombin concentration (Eagle), it follows that prothrombin of the hemophilic plasma is normal in quantity, but is converted to thrombin only with difficulty. The addition of a fixed quantity of platelets accelerates the thrombin production in both solutions markedly, but the hemophilic prothrombin still lags behind the normal prothrombin. However, the amount of thrombin ultimately formed is not significantly affected by the platelets.
5. The Effect of Cephalin upon Hemophilic Blood.—One additional point may be briefly mentioned. In the case of normal plasma, platelets and cephalin are to a certain extent interchangeable: both accelerate the transformation of prothrombin to thrombin, and thus shorten the coagulation time (Eagle). As has just been seen, the retarded coagulation of hemophilic blood is largely due to the retarded formation of thrombin. This can be compensated for by adding an excess of platelets. Cephalin, however, is unaccountably ineffective in accelerating thrombin formation and coagulation in hemophilic blood.

**TABLE III**

*Showing That Cephalin, Unlike Platelets, Does not Markedly Affect the Coagulation of Hemophilic Plasma*

(0.4 cc. of plasma plus varying quantities of cephalin plus 0.2 cc. 0.1 m CaCl₂ plus salt solution to a total volume of 1 cc. Figures in the body of the table represent coagulation time in minutes.)

<table>
<thead>
<tr>
<th>Cephalin suspension (0.2 per cent), cc.</th>
<th>0.4</th>
<th>0.1</th>
<th>0.025</th>
<th>0.0062</th>
<th>0.0031</th>
<th>0.0008</th>
<th>0.0002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human plasma*</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td>17</td>
<td></td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Normal human plasma</td>
<td>5½</td>
<td>4½</td>
<td>6½</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Horse plasma†</td>
<td>15</td>
<td>13</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>23</td>
<td>38</td>
</tr>
<tr>
<td>Hemophilic plasma</td>
<td>120</td>
<td>150</td>
<td>180</td>
<td>240</td>
<td></td>
<td></td>
<td>240</td>
</tr>
<tr>
<td>Hemophilic plasma</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td></td>
<td></td>
<td>&gt;120</td>
</tr>
<tr>
<td>Hemophilic plasma</td>
<td>28</td>
<td>16</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Hemophilic plasma</td>
<td>&gt;60</td>
<td>25</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>

* Platelets not removed.
† Berkefeld filtrate.

(Table III). This puzzling fact, first noted by Mills, requires further investigation, and may provide a clue to the nature of the missing factor in hemophilic plasma.

**SUMMARY**

Despite their reported stability, the platelets of hemophilic blood function normally. The prothrombin content of such plasma is also normal. Confirming the findings of Addis, the delayed coagulation observed in hemophilic blood is due to an unexplained retarded activation of prothrombin to thrombin. The addition of an excess of plate-
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lets, whether normal, hemophilic, or animal in origin, accelerates thrombin production and makes hemophilic blood clot normally; but cephalin, despite the fact that it accelerates thrombin production in normal plasma, is unexplainedly ineffective when added to hemophilic plasma.

BIBLIOGRAPHY


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