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

II. ITS EVOLUTION WITH SPECIAL REFERENCE TO THE INFLUENCE OF CONDITIONS WHICH AFFECT BLOOD COAGULATION

By Benjamin Alexander, M.D., Andre de Vries, M.D.,* and Robert Goldstein, M.D.

With the technical assistance of Eunice Addelson and Elaine Promisel

In a previous communication,¹ a serum factor was described which accelerates the conversion of plasma prothrombin to thrombin by thromboplastin plus calcium, and a method for its determination was reported. This agent, labelled serum prothrombin conversion accelerator (SPCA), is distinct from thrombin, thromboplastin and labile factor. Insufficient data are available to establish the identity or nonidentity of this factor with serum Ac globulin of Ware et al.² or Factor VI of Owren,³ substances with similar physiologic properties.

This paper presents data concerning the evolution of SPCA in human subjects under certain conditions which affect blood coagulation. Similar observations in various hemorrhagic disorders are reported elsewhere in this issue.⁴

METHOD

SPCA was determined by a method based upon the effect of the admixture of serum on the prothrombin time of normal plasma.¹ The activity of SPCA is expressed as the enhancement, in per cent, of the prothrombin activity of a serum-plasma mixture over and above the algebraic sum of the prothrombin activities of each component. Coagulation time of whole blood was determined by a modification of the Lee and White method.⁵

RESULTS

Evolution of SPCA following Coagulation: In previous studies, SPCA was demonstrated in serum obtained from normal blood 1 hour after coagulation.¹ The amount of SPCA which evolves at various intervals after clotting is shown in table 1. Immediately after coagulation, SPCA is low, whereas, as has also been shown by other investigators,⁶ serum prothrombin is high. Within 15 minutes, SPCA increases concomitantly with a decrease in serum prothrombin activity. During the next 45 minutes, some prothrombin activity tends to reappear in the serum, and SPCA activity tends to decrease somewhat.

Normal Variation in SPCA and Serum Prothrombin: The SPCA in 95 normal subjects one hour after blood coagulation varied between 43 and 271 (fig. 1) with a mean of 99.4. The prothrombin activities of the same sera ranged between 0 and 32 per cent.

From the Medical Research Laboratories, Beth Israel Hospital, and the Department of Medicine, Harvard Medical School, Boston.

This study supported by a grant from the Commonwealth Fund.

* Aided by a fellowship from the Rothschild Hadassah University Hospital, Jerusalem.
(fig. 2) with a mean of 6.4. No correlation was evident between the amount of SPCA and the serum prothrombin activity, or the difference in prothrombin ac-

<table>
<thead>
<tr>
<th>Interval after clot</th>
<th>SPCA per cent</th>
<th>Serum proth. activity per cent*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>19</td>
<td>42</td>
</tr>
<tr>
<td>15</td>
<td>95</td>
<td>4</td>
</tr>
<tr>
<td>30</td>
<td>78</td>
<td>11</td>
</tr>
<tr>
<td>60</td>
<td>55</td>
<td>19</td>
</tr>
<tr>
<td>120</td>
<td>65</td>
<td>16</td>
</tr>
<tr>
<td>180</td>
<td>77</td>
<td>15</td>
</tr>
</tbody>
</table>

* The prothrombin activity of normal plasma is considered to be 100 per cent.

Fig. 1

Effect of Accelerating Coagulation: It is well known that agitating freshly drawn blood accelerates coagulation. This procedure also accelerates SPCA evolution, increases the amount of it evolved and decreases residual serum prothrombin activity.
activity (table 2). Defibrination of freshly drawn blood by vigorous shaking yields "serum" which is very rich in SPCA and practically free of prothrombin activity.

The addition of rabbit brain thromboplastin extracts (prepared as for prothrombin determinations from Difco thromboplastin) to blood also accelerates its coagulation, increases in most instances the amount of SPCA* and at the same time renders the serum practically devoid of prothrombin activity (table 3). The question whether this effect of thromboplastin supplements is intimately related to

* Strangely enough, the addition of rabbit brain thromboplastin to freshly drawn dog blood results in decreased SPCA in contrast to the effect on human blood.

---

**Table 2.—Effect of Agitation of Blood on Evolution of SPCA**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Remarks</th>
<th>SPCA (per cent)</th>
<th>Serum prothrombin activity (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Centrifuged and oxalated immed. after coagulation</td>
<td>19</td>
<td>42</td>
</tr>
<tr>
<td>II</td>
<td>Centrifuged and oxalated immed. after coagulation</td>
<td>27</td>
<td>51</td>
</tr>
<tr>
<td>III</td>
<td>The agitated sample was defibrinated by shaking for 8 minutes, which was the clotting time of the non-agitated sample</td>
<td>44</td>
<td>56</td>
</tr>
</tbody>
</table>

* The prothrombin activity of normal plasma is considered to be 100 per cent.
SERUM FACTOR ACCELERATING PROTHROMBIN CONVERSION. II

actual clotting was studied by adding thromboplastin to serum. The addition of thromboplastin to nonoxidated serum increases the amount of SPCA activity in contrast to what obtains with oxidated serum. The serum prothrombin activity was unaffected in one experiment and decreased slightly in another, but was always demonstrable whereas the serum from blood clotted with thromboplastin supplements was always devoid of prothrombin activity.

SPCA has been shown to be distinct from thromboplastin. Nevertheless it is conceivable, in view of evidence that thromboplastin is not consumed during coagulation,7 that the enhancement in SPCA induced by additions of thromboplastin might be related to unconsumed thromboplastin remaining in the serum. Experiments, designed to explore this possibility, revealed that thromboplastin, added to oxidated serum, in proportions comparable to those added to blood not only failed to increase the SPCA activity but in some instances decreased it.*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cl. T.</th>
<th>SPCA</th>
<th>Serum proth. activity</th>
<th>Cl. T.</th>
<th>SPCA</th>
<th>Serum proth. activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without Thromboplastin</td>
<td>12.1</td>
<td>4</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>With Thromboplastin</td>
<td>46</td>
<td>13</td>
<td>&lt;1</td>
<td>141</td>
<td>109</td>
<td>0</td>
</tr>
</tbody>
</table>

* Serum withdrawn and oxidated 1 hour after coagulation.
† Serum withdrawn and oxidated immediately after coagulation.

**Effect of Retarding Coagulation:** Prothrombin activity and SPCA were measured in the serum from blood drawn and allowed to clot in siliconized apparatus according to the technic of Jacques et al.8 Parallel with retardation of coagulation the serum showed abnormally high prothrombin activity and small amounts of SPCA.

The effect of heparin was also investigated. A fixed volume of venous blood was added to increasing concentrations of the anticoagulant (table 4). Although coagulation was retarded substantially in the first two samples, SPCA and prothrombin activity of their sera were unaffected. At larger concentrations of heparin SPCA was markedly reduced and residual prothrombin activity was abnormally high. It appears that the smaller concentrations of heparin increased antithrombin activity without affecting the speed or the amount of prothrombin conversion to thrombin. With larger amounts, this phase of coagulation was also disturbed.

To prove that the above observations were not attributable to heparin carried over into the serum, experiments were performed in which the anticoagulant was added to plasma mixtures in concentrations which would obtain if the serum contained all of the heparin unaltered. The anticoagulant failed to affect substantially the prothrombin activity of the plasma mixtures even in those concentrations

* Thromboplastin added to oxidated dog serum always decreased its SPCA activity.
B. ALEXANDER, A. DE VRIES, AND R. GOLDSTEIN

which retarded coagulation of fresh blood markedly.* It is accordingly evident
that the above observations are not artifacts referable to the mere presence of
heparin in the serum-plasma mixtures upon which prothrombin activities were
determined.

Clot Accelerating Effect of Serum: 2.0 cc of blood from a normal subject were added
to 0.1 cc. of oxalated serum prepared in the usual manner but subjected to incuba-
tion (37 C) for two hours in order to assure maximal inactivation of thrombin.
Its SPCA activity was 173. The clotting time of the normal blood-serum mixture
was 3½ minutes contrasted with a clotting time of the blood alone of 12 minutes.
This clot accelerating effect was not due to thrombin since the same serum added to
oxalated normal plasma (1 part serum to 10 parts plasma) failed to induce clotting
in 3 hours whereas approximately 5 units of thrombin to 1.0 cc. of plasma clotted
the mixture immediately. It is noteworthy that the serum obtained from the whole

<table>
<thead>
<tr>
<th>Hep. added</th>
<th>Cl. T.</th>
<th>SPCA activity</th>
<th>Serum* proth. activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>units per 2 cc. blood</td>
<td>min.</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>59</td>
<td>14</td>
</tr>
<tr>
<td>0.16</td>
<td>12</td>
<td>63</td>
<td>3.3</td>
</tr>
<tr>
<td>0.31</td>
<td>15</td>
<td>93</td>
<td>3.0</td>
</tr>
<tr>
<td>0.63</td>
<td>25</td>
<td>83</td>
<td>8.0</td>
</tr>
<tr>
<td>1.25</td>
<td>39</td>
<td>8</td>
<td>90.0</td>
</tr>
<tr>
<td>1.66</td>
<td>54</td>
<td>13</td>
<td>8.0</td>
</tr>
</tbody>
</table>

* The plasma prothrombin activity of this blood was 90 per cent of normal.

blood-serum mixture whose clotting was accelerated did not show greater SPCA
than the serum from the blood allowed to clot alone.

SPCA in Subjects Receiving Dicumarol: It was of interest to investigate the relation
between the amount of prothrombin available for conversion to thrombin and the
evolution of SPCA. The concentration of this factor was followed in a subject who
received dicumarol for treatment of myocardial infarction.† The administration
and withdrawal of the drug affected plasma prothrombin concentration and SPCA
activity in the same direction (fig. 3). Similar results were obtained in a normal
dog which received dicumarol parenterally.

Hypoprothrombinemic blood from 26 subjects with myocardial infarction who
received dicumarol for treatment‡ was studied. Their plasma prothrombin concen-
trations were between 3.8 and 10 per cent of normal (mean 7.1). The SPCA's ranged
between 8.4 and 43 (mean 25, S.D. 9.7). The serum prothrombin activities were
usually less than that of normal serum, never exceeding 4 per cent.

* It appears that the antithrombic action of heparin in these concentrations does not influence the
prothrombin times markedly.
† This patient showed no additional manifestation of phlebothrombosis or thromboembolism.
‡ Part of a study supported by the U.S.P.H. on the effect of dicumarol on the thrombotic complications
of myocardial infarction.
In view of the observations that thromboplastin added to freshly drawn blood resulted in much greater SPCA, similar experiments were done on hypoprothrombinemic blood from 8 subjects. SPCA was increased substantially in only two instances, although clotting was accelerated not only in these cases but also in those where SPCA was unchanged.

**DISCUSSION**

The ranges of both serum prothrombin conversion accelerator and residual prothrombin activity in serum removed and oxalated one hour after coagulation have been delineated. The explanation for the wide variations is obscure. Two factors just be considered in SPCA evolution: (1) speed of prothrombin conversion to thrombin and (2) the absolute amount of prothrombin converted. From the results obtained with mechanical agitation of, and with thromboplastin supplements to, clotting blood, it is evident that accelerating coagulation increases the amount of SPCA formed. Inhibition of clotting by large amounts of heparin or by siliconized apparatus suppresses SPCA evolution.

It should be pointed out that concomitant with accelerating coagulation more prothrombin is converted to thrombin as evidenced by less prothrombin activity remaining in the serum. Conversely more serum prothrombin is found when coagulation is retarded by silicone or large amounts of heparin. Whether under these conditions the substantial residual serum prothrombin is intimately related to the decreased SPCA or whether it, too, is simply a reflection of the retarded coagulation
requires elucidation. It would appear from experiments on dicumarolized blood that
the total amount of prothrombin converted to thrombin plays an important role in
the total amount of SPCA which can be evolved. This is predicated upon the as-
sumption that dicumarol does not decrease, simultaneously with plasma pro-
thrombin, a precursor of SPCA. The validity of this assumption, however, has yet
to be substantiated.

It is known that the coagulation of dicumarolized blood is prolonged under
certain conditions. That this degree of retardation per se cannot, however,
explain the low SPCA of serum from dicumarolized subjects is indicated by the
inability, in most instances, of increasing SPCA development by accelerating co-
agulation of dicumarolized blood with thromboplastin.

It therefore appears that the amount of prothrombin converted to thrombin is
one of the factors determining the amount of SPCA evolved. Another determinant
is the velocity of prothrombin conversion. Both depend, inter alia, upon the con-
centrations of prothrombin and thromboplastin. That no correlation was evident
between SPCA and the plasma-serum prothrombin activity difference in normal
subjects may be referable to variation from individual to individual in the rate
with which thromboplastin evolves after blood is shed.

An increment in SPCA, similar to that induced by thromboplastin added to
freshly drawn normal blood, can also be produced by adding thromboplastin to
serum which contains small amounts of prothrombin activity. That this enhancing
effect is not obtainable if the serum is oxalated prior to the addition of the thrombo-
plastin suggests that calcium is required for SPCA formation. It is striking that
substantial increments in SPCA are thus obtained although only slight amounts of
additional prothrombin are apparently consumed.

The in vitro action of heparin is of particular interest. Moderate amounts of the
anticoagulant retard coagulation without, however, affecting either the amount
of SPCA evolved or the amount of prothrombin which is consumed during coagula-
tion. If anything, prothrombin consumption is increased, probably as a result of the
greater interval provided by the retarded coagulation for the reaction to proceed.
Although the anticoagulant is said to have antiprothrombic as well as antithrombic
properties, moderate doses seem to act by enhancing the latter. Larger
doses retard coagulation also by inhibiting the evolution of thromboplastin from
platelets or by otherwise preventing the conversion of prothrombin to thrombin.
Concomitant with this, SPCA evolution falls off.

Of fundamental importance is the ability of serum to accelerate the coagulation
of whole blood. Its practical significance derives from the realization that this may
be the mechanism underlying clot propagation in vivo.

Thrombin has been excluded as the clot promoting agent in serum. Since, however,
it has been shown that thromboplastin is not consumed in the process of blood
coaulation, it is possible that the clot accelerating action of serum is due to
unconsumed thromboplastin liberated during blood coagulation. That thrombo-
plastin can thus be implicated is highly unlikely since serum has only slight effect
on the clotting time of hemophilic blood, which is very sensitive to thrombo-
plastin.
The value of dicumarol in the prevention and treatment of thromboembolism may well be related to its interference with SPCA evolution.

**SUMMARY**

1. The evolution of a factor in serum which accelerates prothrombin conversion to thrombin has been studied in normal subjects.
2. Mechanical agitation of fresh blood or the addition of thromboplastin supplements increases the amount of SPCA evolved and decreases the amount of prothrombin activity remaining in the serum.
3. Retarding coagulation by large doses of heparin or by handling the blood with siliconized apparatus decreases SPCA evolution and increases residual serum prothrombin activity.
4. Hypoprothrombinemic blood resulting from dicumarol administration evolves subnormal amounts of SPCA during its coagulation.

**REFERENCES**

A FACTOR IN SERUM WHICH ACCELERATES THE CONVERSION OF PROTHROMBIN TO THROMBIN: II. ITS EVOLUTION WITH SPECIAL REFERENCE TO THE INFLUENCE OF CONDITIONS WHICH AFFECT BLOOD COAGULATION

BENJAMIN ALEXANDER, ANDRE DE VRIES, ROBERT GOLDSTEIN, EUNICE ADDELSON and ELAINE PROMISEL