Nature of the Blood Coagulation Mechanisms in SPCA* Plasma

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Derivatives of purified prothrombin and substances required for the formation of a derivative have been studied in connection with SPCA deficient plasma. The deficiency can be corrected in vitro with prothrombin derivatives. It is concluded that the SPCA patient cannot convert prothrombin to autoprotrombin and thrombin in the same way as a normal individual, and that the precursor of SPCA is prothrombin.

ALEXANDER and associates\(^1\) described a hitherto unrecognized hemorrhagic disease which involved the blood coagulation mechanisms in an unknown manner. The hemorrhagic diathesis could be corrected with the use of serum and serum fractions. They ascribed the therapeutic value of serum to a substance called serum prothrombin conversion accelerator or SPCA. The bleeding tendency has also been diagnosed by others\(^2\) and properties of the serum factor have been extensively described.\(^3\) It has not been obtained in pure form, and its origin has not been adequately clarified. It has been postulated that it arises from a precursor ordinarily found in plasma, and that this precursor is a substance distinctly different from but with many properties quite like prothrombin. Thromboplastin and platelets and calcium ions are believed to play an important role in the activation of the precursor.

This laboratory has conducted an extensive study on the purification of prothrombin,\(^4\) \(^5\) and the prothrombin products obtained are uniformly found to be devoid of the serum prothrombin conversion accelerator.\(^6\) Recently it has been possible to prepare derivatives of prothrombin that possess powerful accelerator activity.\(^11\) One of these derivatives, autoprothrombin, is prepared from prothrombin by adding calcium ions, a purified preparation of platelet factor 3, and a small amount of Ac-globulin to the purified prothrombin. In such a mixture the prothrombin activity disappears rapidly. The preparation is then inert for it cannot be converted to thrombin with any of the known biologic materials commonly associated with the blood coagulation mechanisms. Although this protein, derived from prothrombin, is inert with respect to its capacity to transform to thrombin it possesses powerful accelerator activity.\(^11\) Autoprothrombin accelerates the interaction of prothrombin, calcium ions, thromboplastin and serum Ac-globulin, the latter substance being derived from plasma Ac-globulin by means of thrombin.

Thus we may represent a small segment of the blood coagulation mechanisms by the following equation:

\[
\text{Plasma Ac-globulin} \xrightarrow{\text{Thrombin}} \text{Thrombin} \quad \text{Ca}^{++} \quad \text{Thromboplastin} \quad \text{Serum Ac-globulin} \quad \text{Prothrombin} \quad \text{Autoprothrombin} \quad \text{Thrombin} \quad \text{Ca}^{++} \quad \text{Ac-globulin} \quad \text{Platelet factor 3}
\]

In this paper we show that the slow activation of prothrombin associated with SPCA plasma can be accelerated by autoprothrombin and also by platelet factor 3. This suggests that SPCA, the serum accelerator, is derived from

*SPCA-serum prothrombin conversion accelerator.
prothrombin. In the normal individual prothrombin becomes partly converted to autoprothrombin and partly to thrombin. In the SPCA patient prothrombin is probably not readily converted to SPCA (autoprothrombin) as in the normal individual.

**Procedures**

The materials employed in this work were all prepared by methods previously described; namely, bovine platelet concentrate, purified platelet factor 3, purified prothrombin, citrate-autoprothrombin, and barium carbonate eluate. The analytical methods used for the quantitative determination of prothrombin and Ac-globulin have also been described previously. The plasma from the SPCA patient was kindly supplied by Benjamin Alexander and Robert Goldstein of the Beth Israel Hospital, Boston. The plasma was citrated, dried from the frozen state, and reconstituted to its original volume with distilled water just prior to use in our experiments.

**Results**

In the quantitative determination of Ac-globulin concentration purified prothrombin is used as a substrate. The quantity of thrombin that forms and the rate at which it forms is measured. Without Ac-globulin activation of prothrombin is negligible under the conditions of assay and a unit of Ac-globulin is defined in terms of rigid specifications. In the work described below we made use of an observation recorded by Johnson and Seegers. They found that proconvertin deficient plasma (same as SPCA plasma) gave an apparent Ac-globulin concentration lower than 15 per cent of normal. If a concentrate of the serum factor (BaCO₃ eluate) was supplied in the analytical procedure the Ac-globulin concentration was found to be normal. Their interpretation of the data was that the test indicates, in a semiquantitative manner, whether SPCA is present or not, provided Ac-globulin is not lacking. Whether SPCA is required to overcome an inhibitor under the conditions of the analysis is not known. In our work the observations of Johnson and Seegers were confirmed by analysis of plasma supplied by Alexander and Goldstein. In the analytical procedure the apparent Ac-globulin concentration was 1.8 units per ml. (table 1), which is a much lower concentration than ever found for plasma from healthy individuals.

<table>
<thead>
<tr>
<th>Reagent Added</th>
<th>Units Ac-G/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.8</td>
</tr>
<tr>
<td>BaCO₃ eluate</td>
<td>8.9</td>
</tr>
<tr>
<td>Autoprothrombin</td>
<td>8.6</td>
</tr>
<tr>
<td>Citrate-Autoprothrombin</td>
<td>9.8</td>
</tr>
<tr>
<td>Platelet suspension</td>
<td>9.0*</td>
</tr>
<tr>
<td>Purified platelet factor 3</td>
<td>9.7</td>
</tr>
</tbody>
</table>

* Small correction made for platelet accelerator (platelet factor 1) activity.

BaCO₃ eluate is a preparation obtained from serum and known to be rich in SPCA. It contains a small amount of Ac-globulin, but in the concentrations used in our experiments this involved only a small correction factor. This reagent was added to the SPCA plasma and the analysis for Ac-globulin concentration was then 8.9 U/ml., which is a low value but often found with normal individuals.

Autoprothrombin was prepared from purified prothrombin by adding calcium ions and a purified preparation of platelet factor 3. After autoprothrombin had formed to fullest extent the platelet factor 3 was removed by sedimentation in an ultracentrifuge. When the autoprothrombin was supplied in the analytical procedure for Ac-globulin the SPCA plasma was found to contain 8.6 units of Ac-globulin. The autoprothrombin is thus equivalent to BaCO₃ eluate in this test. Both preparations fully develop the total Ac-globulin titre of the plasma. Since citrate-autoprothrombin is closely related to autoprothrombin in its accelerator properties it was of importance to determine its effect on SPCA plasma.

Citrate-autoprothrombin was prepared from purified prothrombin by dissolving the latter in 25 per cent (w/v) sodium citrate solution. In a short time the prothrombin molecule is degraded in this solution so that multiple components appear upon examination in the electrophoresis apparatus or the ultracentrifuge. Prothrombin itself is not soluble in 7 per cent (w/v) trichloroacetic acid, but after a short while in 25 per cent sodium citrate solution large quantities of nitrogen containing material
become soluble. In due course, the solution acquires thrombin activity, however, the development of this thrombin activity can be inhibited by adding 3,4,4′-triaminodiphenyl sulfone or purified soybean trypsin inhibitor. These blocking agents permit the citrate-autoprothrombin concentration to accumulate and prevent its transformation to thrombin. Citrate-autoprothrombin was prepared with the use of 3,4,4′-triaminodiphenyl sulfone, and was added to SPCA plasma. Then an analysis for Ac-globulin was performed. The result found was 9.9 units Ac-globulin per ml. plasma. This value is comparable to that found with autoprothrombin and with BaCO$_3$ eluate.

In the previous work of Johnson and Seegers it was found that suspensions of disintegrated platelets could be added to SPCA deficient plasma and that the apparent Ac-globulin deficiency of the plasma was corrected. We again performed that kind of experiment and found 9.0 units of Ac-globulin with our sample of plasma. Since it is specifically platelet factor 3 which is needed to convert prothrombin to autoprothrombin it was important to see whether this factor could be used to develop the Ac-globulin titre of the plasma. It was found that this occurs with our purified preparation of platelet factor 3. The plasma Ac-globulin concentration was found to be 9.7 units per ml. though the preparation of purified platelet factor 3 itself does not contain any Ac-globulin or autoprothrombin.

**DISCUSSION**

As in the work of Seegers and Johnson we find that an analysis of SPCA plasma for Ac-globulin gives low values and these are restored to normal if platelets or BaCO$_3$ eluate are supplied. Furthermore, platelet factor 3, autoprothrombin or citrate-autoprothrombin serve the same purpose. We believe that autoprothrombin, a substance derived from prothrombin, supplies the deficiency represented by SPCA plasma. When platelets or platelet factor 3 are used to correct the deficiency in our Ac-globulin analysis the autoprothrombin is formed from the patient’s own prothrombin by means of platelet factor 3 as follows:

\[
\begin{align*}
\text{Ca}^{++} & \quad \text{Platelet factor 3} \\
\text{Prothrombin} & \quad \text{Ac-globulin} \quad \rightarrow \quad \text{Autoprothrombin}
\end{align*}
\]

The data are consistent with the view that the SPCA patient cannot convert prothrombin to autoprothrombin in the same way as a normal individual. The reasons for this inability are not known.

Alexander, Owren, Koller, Loeliger and Duckert and others found the accelerator needed by the SPCA patient to be in normal serum. They postulate a precursor in plasma called proconvertin, pro-SPCA, etc. They have made attempts to devise quantitative methods for measuring the activity of this precursor and have suggested mechanisms for its activation. We do not believe that their viewpoints regarding the precursor can be upheld despite the extensive literature. We regard the postulated precursor of the serum factor as prothrombin itself. Their conclusions are actually extrapolations that reach a little far for critical discernment. On the basis of the procedures they employed their data can be given another interpretation. It is interesting, for example, that the methods of Owren were so non-specific and precarious that a complete transposition of the words “prothrombin” and “proconvertin” were made in a manuscript. A more accurate correction would have been to say that prothrombin and proconvertin are the same. It is quite significant that no one has been able to prepare a concentrate of the hypothetical precursor and show that it is different from prothrombin.

Owren has applied his methods for the study of obstructive jaundice. The hypothetical precursor of the accelerator is said to decrease in concentration parallel with the decrease in prothrombin concentration. This observation corresponds with our view that prothrombin and the precursor are one and the same substance. In this connection it is helpful to consider the experiments of Lasch and Roka who were able to obtain prothrombin from a serum precursor by means of liver mitochondria. It is
thus possible to convert prothrombin to a derivative and to convert a derivative found in serum to prothrombin.

Some authors\(^6\) believed that an activator of prothrombin decreases in concentration in plasma before prothrombin concentration drops appreciably when dicumarol is given. Apparently this activator is supposed to be identical with proconvertin, pro-SPCA, etc. This does not necessarily follow from data thus far presented. The facts are compatible with the suggestion which we offer for the first time; namely, that the accelerator deficient in dicumarol plasma is more than a deficiency in prothrombin and its derivative autoprothrombin. The latter could hardly be found in dicumarol serum in normal concentration if prothrombin itself decreased in concentration. Very likely, when more data become available, it will be recognized that quite another accelerator that is not related to autoprothrombin decreases in concentration in dicumarol plasma.

It is interesting, for example, that Johnson and Seegers\(^10\) wrote: "Thus material adsorbed on BaCO\(_3\) and eluted with sodium citrate contains very little if any Ac-globulin itself, but can enable proconvertin deficient plasma to develop its full Ac-globulin titre. Platelet extracts develop a similar titre in proconvertin deficient plasma. By contrast with these experiments our method for Ac-globulin analysis gives normal values for dicumarol plasma without addition of platelet extract and BaCO\(_3\) eluate preparation. Nevertheless dicumarol plasma is supposed to be deficient in proconvertin." Further study of the properties of dicumarol plasma are indicated from the newer knowledge of the properties of prothrombin and its derivatives.

**Summary**

When SPCA deficient plasma is analyzed for its Ac-globulin content it apparently contains only a small amount. The full titre can, however, be found if the analytic procedure is modified by adding autoprotrombin, citrate-autoprothrombin, platelets, platelet factor 3, or a preparation containing SPCA. Since autoprotrombin is derived from prothrombin by means of platelet factor 3, and since it can supply the same activity as an SPCA concentrate from serum the two are regarded as the same. The precursor of SPCA is considered to be prothrombin and it is believed that the SPCA patient cannot convert prothrombin to autoprotrombin and thrombin in the same way as a normal individual.

**REFERENCES**


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