Starch Block Electrophoresis of Plasma and Serum Clotting Factors. Separation of Activated PTC (PTC')

By Sandra Schiffman, Samuel I. Rapaport and Jane Patch

Paper strip and paper curtain electrophoretic technics have been widely used to separate or identify clotting factors. Recently, starch gel electrophoresis has also been employed. We report herein the results of starch block electrophoresis using starch thoroughly washed with ethylenediaminetetraacetate (EDTA).

We have characterized the migration pattern of the following plasma clotting factors: Hageman factor (XII), plasma thromboplastin antecedent (PTA, XI), proconvertin (VII), prothrombin (II), plasma thromboplastin component (PTC, Christmas factor, IX), and Stuart factor (X). These factors were recovered in an estimated 30–55 per cent of original plasma activity.

In addition to these native plasma factors, two intermediates of intrinsic coagulation were isolated. Activated PTA (PTA') was eluted from both plasma and serum starch blocks. Activated PTC (PTC') was recovered only from serum blocks. PTC' was found to migrate more slowly than native PTC, evidence that activation is associated with a physical change in this molecule.

Materials

Intact normal plasma. Venous blood was drawn through a Monocote E (Armour Laboratories)-coated needle into a silicone (G.E. SC-87, Dri-film)-coated syringe and mixed with anticoagulant in a ratio of 9 parts of blood to 1 part of anticoagulant. The anticoagulant was a solution 0.06M in trisodium citrate and 0.04M in citric acid. The blood was centrifuged in plastic tubes for 10 minutes at 12,500 g. at 3 C. to obtain the supernatant platelet-poor plasma. Plasmas were either used fresh or frozen in small aliquots in tightly capped, 1.2 ml. plastic vials at −20 C.

Two-hour glass serum. Blood was drawn by silicone technic as described above, and about 5 ml were added to each of several 16 x 100 mm. glass tubes containing 5 glass beads. The tubes were inverted 5 times and then allowed to incubate at 37 C. for 2 hours. The clots were removed by centrifugation and 1 ml of the citrate anticoagulant described above was added to each 5 ml of serum. The citrated serum was either used immediately or stored at −20 C. in plastic vials.

Activated serum. Blood was drawn by silicone technic as described above into a chilled syringe, transferred to a chilled plastic tube, and centrifuged at 100 g. for 20 minutes at 3 C. The supernatant platelet-rich plasma was removed and stored at 3 C. The cells were respun at 1500 g. for 5 minutes and the additional platelet-poor plasma was added to the platelet-rich plasma. The mixed plasma was then poured into a plastic test tube containing 10 mg./ml. diatomaceous earth powder Dicalite 4200 (Great Lakes Car...
bon Co., Walteria, Calif.) and tilted at 37 C. until it clotted. About 3 minutes later the
clot was compressed with a wooden applicator stick and another 10 mg./ml. of Dicalite
4200 added. After 1 hour at 37 C., anticoagulant was added in a ratio of 1 volume to 5
volumes of serum, and the mixture, with the clot removed, was centrifuged for 10 min-
utes at 12,500 g. The “activated serum” was either used immediately or stored at −20 C.
in plastic vials.

Barbital buffer is a solution of 0.028M NaCl plus 0.125M sodium barbital. The pH is
adjusted to 7.25 with 0.1N HCl.

Cephalin is an acetone-insoluble, ether-soluble extract of human brain. It was diluted,
usually 1/100, in barbital buffer before addition to clotting mixtures.

Citrate-saline is a solution of one part 0.1M trisodium citrate and five parts 0.85 per
cent sodium chloride.

METHODS

Electrophoresis. Starch (Baker Purified Potato Starch Powder, No. 4008) was washed
by shaking and decanting with approximately the dry powder volume of the following
solutions: four times in distilled water, four times in 0.05M disodium ethylenediaminetetra-
acetate (EDTA) adjusted to pH 8.6 with NaOH, one time in citrate-saline, and finally
two times in a solution of five parts 0.05M sodium barbital adjusted to pH 8.6 with HCl
and one part 0.1M trisodium citrate. The washed starch was then suspended in a mini-
mum volume of the final washing solution and poured into a starch block form 34 x 7.5
x 1.5 cm. using two pourings. The surface was blotted each time with paper towels,
and then the block was wrapped in Saran-Wrap and placed in a cold room overnight. A 3 ml.
sample was mixed with washed starch, air dried on paper towels to a consistency slightly
more moist than the block, and inserted into a trough cut in the block. Wet sponges con-
nected the block to electrode vessels containing a solution of one part 0.1M trisodium
citrate and five parts of a solution 0.1M in NaCl and 0.05M in sodium barbital adjusted
to pH 8.6. The block was allowed to run for 24 hours in a cold room. Initially the voltage
was set at about 100V with about 40 ma. current. On occasion the current rose during
the course of the run. At the end of 24 hours the block was cut into 1 cm. sections which
were eluted with 3 ml. of citrate-saline. Highest yields were obtained by forcing
the eluting fluid through the starch. A section of starch was placed in a plastic test tube
with a perforated bottom covered by filter paper. This tube was then suspended in a
larger tube, eluting fluid was added, and the combination was gently centrifuged. About
4 ml. of eluate was recovered from the bottom of the larger tube. It was dialyzed over-
night in a cold room against citrate-saline and then used immediately or stored in plastic
vials at −20 C.

Clotting Factor Assays

1. Assays to Detect PTC'

(a.) The intact PTC assay was carried out in the absence of an activating surface so
PTA' which usually triggers intrinsic clotting was not generated in the mixture. There-
fore, a test substance exhibiting significant activity in this system must contain either a
combination of PTA' and native PTC, PTC', or an active intermediate of clotting beyond
PTC'. Thrombin was added to the substrate to eliminate any effect of a trace of thrombin
in a test substance which may otherwise cause nonspecific shortening. The test was per-
formed as follows: to 0.1 ml. of cephalin 1/100 in a plastic clotting tube were added 0.1
ml. of PTC deficiency plasma and 0.1 ml. of 0.1 U/ml. of thrombin. Exactly 3 minutes
later, 0.1 ml. of 40mM CaCl2 was added followed immediately by 0.1 ml. of test sub-
stance. Siliclad-coated pipettes were used throughout. Clotting time was measured from
the addition of the test substance. Blank time ranged from 5 to 8 minutes.

(b.) The PTC' generation assay is designed to detect native PTC in a test substance
by measuring its ability to be activated to PTC' in the presence of PTA' and calcium.31
A PTC preparation which does not activate in this system is, by definition, PTC'. Thrombin was again added to the substrate for the reason given above. The test was performed as follows: PTA', test substance, and 0.315M CaCl₂ in a ratio of 1:1:0.1 were incubated at 37°C. At specific intervals 0.1 ml. of this mixture was subsampled into a second tube containing 0.05 ml. of cephalin 1/50, 0.1 ml. of PTC deficiency plasma, and 0.05 ml. of 0.4 U/ml. of thrombin, and the clotting time was noted. This second mixture incubated exactly 3 minutes at 37°C before the aliquot from the first mixture was added to it. All procedures were carried out in plastic tubes using Siliclad-coated pipettes. Blank times in this assay exceed 250 seconds.

2. Other Assays
   Assays for native PTC (contact PTC assay), Hageman factor, and PTA were based on a modified partial thromboplastin time with kaolin system. Thrombin activity was measured by adding 0.1 ml. of test substance to 0.2 ml. of fibrinogen solution (Warner-Chilcott) made up to 2.00 mg. per cent in citrated, isotonic saline. Siliclad-coated pipettes and plastic clotting tubes were used. The test was considered negative if no fibrin threads were observed in 18 to 24 hours. Detailed conditions for the determination of PTA', (described as the preformed activation product assay), proconvertin, and for the thromboplastin generation test have been described elsewhere. Antihemophilic globulin (AHG, VIII) was measured by incubating 0.1 ml. of a suspension of kaolin 10 mg/ml. in cephalin 1/100, 0.1 ml. of AHG deficiency plasma, and 0.1 ml. of test substance for 9 minutes at 37°C and then recalcifying with 0.1 ml. of 40 mM CaCl₂. Stuart factor was measured by a slight modification of Hougie's technic. Proaccelerin (V) was measured by the method of Stormorken. Clotting factor activities are reported as per cent of one standard normal plasma unless noted otherwise.

   Protein was estimated by absorbancy at 280 μm.

RESULTS

The behavior of clotting factors on starch block electrophoresis depends upon the washing procedure. The migration patterns reported herein are obtained only if the starch is washed with EDTA. Otherwise thrombin is generated on the starch, and plasma clotting factors cannot be recovered from citrated plasma. Formation of thrombin has also been reported during paper electrophoresis.

The results of electrophoresis of intact normal plasma are shown in figure 1. Clotting factor activities are found in two general areas: (1) the origin area, which contains the contact activation factors, and (2) the α-globulin to albumin area, which contains the vitamin K-dependent factors. No AHG, proaccelerin, or thrombin was recovered.

The contact activation factors—Hageman factor, PTA, and activated PTA (PTA')—cluster between the γ- and β-globulins, making their separate identification difficult. Moreover, since PTA', by definition, can correct the defect in both PTA and Hageman factor assay systems, one has the added problem of distinguishing apparent from real PTA and Hageman factor activities. Nevertheless, it was possible to locate all three activities.

In four plasma starch blocks native PTA appeared to precede both PTA' and Hageman factor. In figure 1 a definitive PTA peak migrates behind PTA' and Hageman factor. In the three other plasma blocks this distinct peak was
STARCH BLOCK ELECTROPHORESIS OF CLOTTING FACTORS

![Electrophoresis Pattern of Normal Plasma](image-url)

**Fig. 1.—The electrophoretic pattern of normal plasma.** Clotting factor activities are reported as per cent of a specific normal plasma except for PTA' which is based on an arbitrary standard preparation since little or no PTA' is present in normal plasma.

not observed; however, a disproportionate rise in PTA activity in the very early PTA' tubes strongly suggested the presence of native PTA preceding the main PTA' zone.

Identification of PTA' posed no problem since this activity specifically corrects the defect in exhausted plasma\(^{20,21}\) in the absence of an activating surface. However, the accompanying PTA and Hageman activities (fig. 1) could be either real or apparent. Since native PTA had been identified preceding the PTA' peak it seems unlikely that another native PTA peak migrates with the PTA'. However, this possibility cannot be excluded.

The presence of Hageman factor in the PTA' peak was strongly suspected because starch block fractions of PTA' clotted much more rapidly in the Hageman assay than did comparable strength PTA' made by chromatography on a diatomaceous earth column. For example, a starch block fraction which clotted in the PTA' assay in 70 seconds clotted in the Hageman assay in 47 seconds; a PTA' preparation from a diatomaceous earth column which gave the same clotting time in the PTA' assay clotted in the Hageman assay in 108 seconds. The presence of Hageman factor in starch block fractions was confirmed by DEAE-cellulose chromatography. On four occasions, portions of several starch block tubes containing PTA' and apparent Hageman factor were pooled and applied to a small DEAE column using a technic described earlier.\(^{11}\) Under these conditions PTA' elutes at 0.05M salt concentration whereas Hageman factor eluates at about 0.18M salt concentration. Therefore, if both factors were present in the pooled fraction each should elute as a separate activity. As the data in table 1 show, PTA' activity was found in the
Table 1.—The Results of DEAE Chromatography of a PTA'—Hageman Factor Area of a Starch Block

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Hageman Assay (sec.)</th>
<th>PTA' Assay (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05M (PTA') area</td>
<td>145</td>
<td>67</td>
</tr>
<tr>
<td>0.18M (Hageman) area</td>
<td>55</td>
<td>97</td>
</tr>
<tr>
<td>Blank</td>
<td>330</td>
<td>140</td>
</tr>
</tbody>
</table>

0.05M salt region followed by Hageman activity in its expected position.

It was simpler to identify the vitamin K-dependent factors since separations were fairly distinct on the starch blocks and each factor corrected only its specific assay. Proconvertin migrates predominantly with the α₂-globulins, a large portion of it uncontaminated with other clotting activities. Prothrombin migrates with the α₁-globulins, and the maximum Stuart factor and PTC activities coincide with the albumin peak. They could not be separated by this technic.

The electrophoretic pattern of "2-hour glass serum," shown in figure 2, resembles the pattern of plasma in that the clotting factors are concentrated around the origin and the α-globulin to albumin regions. Thrombin was not eluted.

The origin area contains the same clotting factors as the plasma block, PTA, Hageman factor, and PTA'. PTA migrates distinctly behind PTA' and Hageman factor. Again evidence for Hageman factor in the PTA' zone is provided by shorter clotting times in a Hageman assay than could be accounted for by PTA' alone. For example, a sample from the origin tube clotted in the PTA' assay in 95 seconds and in the Hageman assay in 50 seconds. A clotting time in the Hageman assay of about 150 seconds would be expected with PTA' of the same strength from a diatomaceous earth column. However, confirmation of the presence of Hageman factor by DEAE-cellulose chromatography could not be obtained. Whenever active fractions from a serum block were pooled and applied to a column only trace activities could be recovered. Both the PTA' and the Hageman factor activities from serum appear to be more labile than those from plasma.

The major apparent difference between serum and plasma blocks is seen in the α-globulin to albumin region. As in plasma, proconvertin migrates with the α₂-globulins, and native PTC and Stuart factor migrate with albumin. No prothrombin is recovered. However, a new activity is eluted in the α₁-globulin region which corrects the defect in PTC deficiency plasma in the absence of an activating surface. Recently an activated form of PTC, PTC', has been described which has this clotting property. Since PTC' is synthesized rapidly in the presence of PTA' and calcium,¹¹,²² both of which are present during the preparation of "2-hour glass serum," it seemed likely that the new activity was in fact PTC'. Therefore, this activity, measured in the intact PTC assay, is designated PTC' in figure 2. However, at least two other possibilities had to be considered: (1) a combination of native PTC and PTA', or (2) an intermediate of intrinsic prothrombinase synthesis beyond PTC' such as activated Stuart factor²³ or autoprosthrombin C.²⁴
Fig. 2.—The electrophoretic pattern of “2-hour glass serum.” Clotting factor activities are reported as per cent of a specific normal plasma except for PTA’ which is based on an arbitrary standard preparation and PTC’ which is measured as per cent of the original serum activity in the intact PTC assay. An apparent PTA’ peak which coincided with the PTC’ peak is not included in the figure to avoid confusing points.

A peak containing both PTA’ and native PTC seemed highly unlikely because PTA’ had already been identified at the origin, and native PTC had already been identified in the albumin area. One would have to postulate a second peak of each with the same activity but a different mobility. The presence of native PTC was ruled out by showing that a fraction containing apparent PTC’ activity failed to generate added PTC’ when mixed with PTA’ and calcium. The difference in behavior in the PTC’ generation assay between this apparent PTC’ and native PTC is shown in table 2.

The alternative possibility, an active intermediate other than PTC’, could not be ruled out easily using “2-hour glass serum.” Therefore, “activated serum” was made as described in the MATERIALS section. In this preparation, diatomaceous earth powder is added to produce maximum PTA’, which should facilitate the synthesis of subsequent in vitro intrinsic clotting intermediates.

The electrophoretic pattern of “activated serum” is shown in figure 3. A striking change is noted in the migration of the PTC activity measured in

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>PTC' Generation Assay*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0'</td>
</tr>
<tr>
<td>Tube 12 (apparent PTC')</td>
<td>150''</td>
</tr>
<tr>
<td>Tube 15 (native PTC)</td>
<td>82''</td>
</tr>
</tbody>
</table>

*See details in Methods section.
Fig. 3.—The electrophoretic pattern of “activated serum.” Activities reported as described under figure 2. For clarity the PTA and Hageman factor activities of tubes 17 through 20 are not shown. The apparent Hageman, PTA, and PTA′ activities in this region are interpreted as primarily a reflection of PTC′ activity which can partially correct the defect in the three assay substrates.

The contact assay. Instead of migrating with the albumin peak, as in plasma (fig. 1) and “2-hour glass serum” (fig. 2), this PTC contact activity is now associated with the \( \alpha_1 \)-globulins and coincides with the PTC′ peak measured in the intact PTC assay. The migration of Stuart factor with albumin is unchanged. Since the apparent PTC′ activity from “activated serum” has both intact and contact PTC activity without Stuart factor activity, it cannot be activated Stuart factor. Furthermore, in contrast to autoprothrombin C, these fractions fail to correct an AHG assay. Neither activated Stuart factor nor autoprothrombin C can therefore account for the observed activity.

Again it would be difficult to explain this activity as combined PTA′-native PTC for the reasons mentioned above. However, to prove the point these fractions were tested for native PTC in the PTC′ generation assay. Table 3 shows the reactions with PTA′ and calcium of several fractions from the block.

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>PTC Assay</th>
<th>PTC′ Generation Assay*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact</td>
<td>Intact</td>
</tr>
<tr>
<td>Tube 15</td>
<td>68°</td>
<td>26°</td>
</tr>
<tr>
<td>Tube 16</td>
<td>63°</td>
<td>22°</td>
</tr>
<tr>
<td>Tube 17</td>
<td>57°</td>
<td>20°</td>
</tr>
<tr>
<td>Tube 18</td>
<td>53°</td>
<td>19°</td>
</tr>
<tr>
<td>Tube 20</td>
<td>62°</td>
<td>38°</td>
</tr>
<tr>
<td>Blank</td>
<td>145°</td>
<td>&gt;360°</td>
</tr>
</tbody>
</table>

*See details in Methods section.
Table 4.—A Comparison of PTC' Activity in “2-Hour Glass Serum” and “Activated Serum”

<table>
<thead>
<tr>
<th></th>
<th>2-Hour Serum Intact PTC Assay</th>
<th>Activated Serum Intact PTC Assay</th>
<th>Act. Serum/2-Hr. Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(sec.)</td>
<td>%</td>
<td>(sec.)</td>
</tr>
<tr>
<td>Plasma</td>
<td>120</td>
<td>0.09</td>
<td>102</td>
</tr>
<tr>
<td>Serum</td>
<td>43*</td>
<td>6.75</td>
<td>36†</td>
</tr>
<tr>
<td>Peak PTC'</td>
<td>50</td>
<td>0.9</td>
<td>23</td>
</tr>
<tr>
<td>Blank</td>
<td>&gt;360</td>
<td></td>
<td>&gt;360</td>
</tr>
</tbody>
</table>

* Diluted 1/5 in citrate-saline.
† Diluted 1/50 in citrate-saline.

illustrated in figure 3. The early PTC' tubes, which contained powerful intact PTC activity, could not generate additional PTC'. Therefore, they must contain PTC'-like activity without native PTC. As the fractions approached the albumin peak they acquired the ability to generate PTC'. This is interpreted to represent a relatively small amount of native PTC migrating with the albumin peak as observed in plasma and “2-hour glass serum” blocks.

To summarize, the apparent PTC' fractions corrected PTC and to a lesser extent PTA and Hageman assays; had no significant activity in Stuart factor, AHG, proaccelerin, prothrombin, or proconvertin assays; contained no thrombin; and could not be explained as a combination of PTA'-native PTC activities. Therefore, we conclude that the activity measured in the intact PTC assay is in fact PTC', and that PTC' migrates as an α1-globulin whereas native PTC migrates with albumin.

Figures 2 and 3 do not reveal the dramatic increase in absolute amount of PTC' found in “activated serum” because the per cent PTC' shown in each graph is the per cent of that present in the starting serum. To compare the PTC' activity of “2-hour glass serum” and “activated serum” the clotting times of each in the PTC intact assay were read off of a single curve made with “activated serum” as the standard. Since these data were obtained on different days and since the assay varies from day to day (probably because of slight differences in the thrombin added to the substrate), it was necessary for this comparison to assume that the intact PTC activity of the starting plasmas was the same. The percentages obtained from the “activated serum” curve were corrected accordingly. Typical data for the two types of sera and their peak PTC' tubes are shown in table 4. It can be seen that “activated serum” contained 7 to 10 times the PTC' activity of “2-hour glass serum.”

The effect of fractions containing either predominately native PTC or only PTC' upon a two stage thromboplastin generation system using PTC deficiency serum is shown in table 5. The data show that both fractions corrected the defective thromboplastin generation of PTC deficiency serum. PTC' appeared to be more effective when two fractions, containing close to the same apparent amount of contact PTC activity, were used. Maximum activity generated more rapidly with PTC' fractions than with native PTC fractions.

The “activated serum” block exhibited one other very interesting feature. A second apparent PTA' peak migrated between the origin and proconvertin
Table 5.—The Effect of Native PTC and PTC' on the Thromboplastin Generation Test

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>PTC Assay</th>
<th>Thromboplastin Generation Test*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractions</td>
<td>Contact</td>
<td>Intact</td>
</tr>
<tr>
<td>12 (PTC')</td>
<td>73&quot;</td>
<td>45&quot;</td>
</tr>
<tr>
<td>19 (native PTC)</td>
<td>71&quot;</td>
<td>107&quot;</td>
</tr>
<tr>
<td>14 (PTC')</td>
<td>75&quot;</td>
<td>33&quot;</td>
</tr>
<tr>
<td>21 (native PTC)</td>
<td>80&quot;</td>
<td>97&quot;</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>75&quot;</td>
</tr>
</tbody>
</table>

*The incubation mixture consisted of 0.2 ml. aliquots of: Al(OH)₃ adsorbed plasma 1/5 in barbital buffer, PTC deficiency serum 1/10 in barbital buffer, cephalin 1/100, test substance, and 30 mM CaCl₂. At the intervals shown above 0.1 ml. of 20 mM CaCl₂ and 0.1 ml. of the incubation mixture were added to 0.1 ml. of normal plasma and the clotting time noted.

\* Tested at 4 not 3 minutes.

which could not be explained in terms of any other known clotting factors besides PTA'. The differentiation between this peak and the other PTA' peak at the origin awaits further experimentation.

**DISCUSSION**

In our experience starch carefully washed with EDTA has proved to be a good supporting medium for electrophoretic separation of several clotting factor proteins. The only detected change in clotting activities caused by the isolation procedure was the partial activation of PTA. The following plasma clotting factors were found to elute in measurable quantities: Hageman factor, PTA, proconvertin, prothrombin, Stuart factor, and PTC. For purposes of discussion these factors may be divided into two groups: (1) the contact activation group which includes Hageman factor, PTA, and activated PTA (PTA'), and (2) the vitamin K-dependent factors—prothrombin, proconvertin, Stuart factor, and PTC.

The mobility of the contact activation factors on starch block electrophoresis agrees with that reported for paper and paper curtain electrophoresis. Both PTA\(^*$\) and Hageman factor\(^*$\) have been designated either as \(\beta\)-globulins or as migrating between the \(\gamma\)- and \(\beta\)-globulins. "Glass factor," reflecting PTA' activity, also migrated in this region on paper curtain. On starch block we found all three activities between the \(\gamma\)- and \(\beta\)-globulins in the area of the origin. Within this region the mobilities of PTA and Hageman factor can be differentiated. PTA appears to be closest to the \(\gamma\)-globulins, followed by a peak containing both Hageman factor and PTA'.

The mobilities of the vitamin K-dependent clotting factors seem to be much more sensitive to electrophoretic conditions. A summary of the results is shown in table 6. There are two additional studies on starch gel electrophoresis in which only the order of migration was shown. In one, the increasing order of mobility is proconvertin, PTC, Stuart factor, whereas in the
STARCH BLOCK ELECTROPHORESIS OF CLOTTING FACTORS

Table 6.—Results of Electrophoresis of the Vitamin K-dependent Clotting Factors

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Medium</th>
<th>Proconvertin</th>
<th>Prothrombin</th>
<th>PTC</th>
<th>Stuart Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Owen⁵</td>
<td>Paper</td>
<td>β</td>
<td>α₂</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2. Aggeler²</td>
<td>Paper</td>
<td>—</td>
<td>β₂</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3. Gouttas⁵</td>
<td>Paper</td>
<td>β₂</td>
<td>α₁-alb.</td>
<td>β</td>
<td>—</td>
</tr>
<tr>
<td>4. Fisch⁶</td>
<td>Paper</td>
<td>α₁β</td>
<td>α₁-alb.</td>
<td>α</td>
<td>α₁-alb.</td>
</tr>
<tr>
<td>5. Lewis⁷</td>
<td>Paper Curtain</td>
<td>α₁₁-alb.</td>
<td>α₁-alb.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6. Johnston⁸</td>
<td>Paper Curtain</td>
<td>α₁₁-alb.</td>
<td>α₁₁-alb.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7. Lanchantin²⁵</td>
<td>Starch Block</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8. Raber⁶</td>
<td>Starch Block</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>α and β</td>
</tr>
<tr>
<td>9. Bidwell²⁷</td>
<td>Cellulose Acetate</td>
<td>—</td>
<td>β-α₂</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10. Present Study</td>
<td>Starch Black</td>
<td>α₂</td>
<td>α₁</td>
<td>alb.</td>
<td>alb.</td>
</tr>
</tbody>
</table>

other the order is prothrombin, proconvertin, Stuart factor.⁹ In the former a serum reagent was used so prothrombin was not determined.

In our present study using starch block electrophoresis the vitamin K-dependent factors of plasma migrated between the α-globulins and albumin as shown in table 6, line 10. Proconvertin, migrating with the α₂-globulins, could be obtained essentially free of the other three, and a combined native PTC-Stuart factor fraction, migrating with albumin, could be recovered which contained little or no prothrombin and no proconvertin.

Electrophoresis of serum revealed no change in the relative mobility of proconvertin or Stuart factor. However, a new activity which we have identified as activated PTC (PTC') was found migrating in the α₁-globulin region occupied by prothrombin in plasma. PTC' could be most clearly demonstrated using “activated serum” which is prepared in the presence of greatly increased amounts of PTA'. This PTC' has the following characteristics: it corrects the defect in PTC deficiency plasma in a one-stage assay in the absence of an activating surface, it corrects the defect in PTC deficiency serum in a thromboplastin generation assay, and it cannot be further activated upon addition of PTA' and calcium.

Starch block electrophoresis allowed us not only to identify PTC' in serum but also to separate it from Stuart factor, prothrombin, and native PTC. The difference in mobility between native and activated PTC provides evidence that a physical change has occurred during activation.

SUMMARY

1. Plasma clotting factors separate into two groups on starch block electrophoresis. The contact activation factors—Hageman factor, PTA, and activated PTA—remain around the origin, whereas the vitamin K-dependent factors—prothrombin, proconvertin, Stuart factor, and PTC—migrate between the α-globulins and albumin. AHG, proaccelerin, and thrombin are not recovered.

2. The electrophoretic pattern of serum differs from that of plasma mainly in the absence of prothrombin and in the presence of activated PTC (PTC').

3. The electrophoretic mobility of PTC' is found to differ from that of native PTC. This difference may be exploited to separate PTC' from its native form and from Stuart factor.
SUMMARIO IN INTERLINGUA

1. Le factores coagulatori del plasma se separa, in le electrophorese a bloco de amylo, in duo gruppos. Le factores a activation per contacto—le factor Hagemann, PTA (=Antecedente de Thromboplastina del Plasma), e PTA activate—remane in le vicinitate del origine durante que le factores a dependientia de vitamina K—prothrombina, proconvertina, factor Stuart, e PTC (=Componente Thromboplastinic del Plasma)—migra inter le globulinas α e albumina. AHG (=Globulina Anti-Hemolytic), proaccelerina, e thrombina non es recovrate.

2. Le configuration electrophoretic de sero differe abillo de plasma primarimente in le absentia de prothrombina e in le presentia de activate PTC (i.e., PTC').

3. Esseva trovate que le mobilitate electrophoretic de PTC' differe ab illo de PTC native. Iste differentia pote esser exploitate in separar PTC' ab su forma native e ab factor Stuart.

REFERENCES


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Starch Block Electrophoresis of Plasma and Serum Clotting Factors. Separation of Activated PTC (PTC')

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