The Mechanism of Action of Human Accelerator Globulin and Its Relation to Other Clotting Factors

By Merle Lovell Lewis, Ph.D. and Arnold G. Ware, Ph.D.

The concept of blood coagulation has undergone drastic revision in the past decade. A number of different names have been given to the substances in serum and plasma which accelerate the conversion of prothrombin to thrombin. In an effort to resolve some of the resultant confusion in nomenclature, the Josiah Macy Conference group on blood clotting and allied disorders appointed a sub-committee comprised of five of the leading workers in the field. Their object was to arrive at some agreement regarding the identity or nonidentity of various factors involved in the conversion of prothrombin to thrombin. The group recognized two main plasma accelerators, each of which was converted to a more potent accelerator in serum. One of these factors, plasma Ac-globulin or proaccelerin (labile factor, prothrombin accelerator, plasma prothrombin accelerator, plasmatic cofactor of thromboplastin), is converted during spontaneous coagulation or under the influence of thrombin to the more active serum Ac-globulin or acceberin. It has been described by various investigators as unstable, not adsorbed on barium sulfate, not influenced by the administration of dicumarol, and occurring in the plasma fraction precipitated between one-third and one-half saturation with ammonium sulfate. The second accelerator, proconvertin or SPCA precursor (co-factor V, co-thromboplastin, factor VII), is converted during the course of coagulation to a more active substance, convertin or SPCA. SPCA is said to be stable, adsorbed on barium sulfate, decreased by the administration of dicumarol; the presence of Ac-globulin is said to be necessary for SPCA to exert its accelerator action.

The present investigation was undertaken in an effort to elucidate the mechanism of action of human Ac-globulin (Ac-G). The results indicate that there may be actually only one accelerator system in human plasma, rather than two. The evidence strongly suggests that plasma Ac-globulin is converted by thrombin to the much more active serum Ac-globulin, most of which is then rapidly inactivated by an inhibitor.

There is also evidence to suggest that under certain conditions there may be a change in the prothrombin molecule, causing it to become more easily converted to thrombin.

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We wish to acknowledge the technical assistance of Mrs. Irene M. Smyth. We also wish to express our appreciation to Dr. Fremont E. Davis, Miss Angelyn A. Konugas, and the staff of the Transfusion Laboratory, Los Angeles County Hospital, for their cooperation in obtaining the blood used in these experiments.
MERLE LOVELL LEWIS AND ARNOLD G. WARE

Table 1.—Activation of Plasma Ac-G by Thrombin

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Ac-G activity</th>
<th>% of Std.*</th>
<th>Thrombin added</th>
<th>After conversion</th>
<th>Dilution</th>
<th>Ac-G activity</th>
<th>% of Std.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seconds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1:20</td>
<td>37.7</td>
<td>100</td>
<td>10</td>
<td>1:200</td>
<td>37.0</td>
<td>1070</td>
</tr>
<tr>
<td>2</td>
<td>1:20</td>
<td>35.7</td>
<td>125</td>
<td>10</td>
<td>1:200</td>
<td>35.7</td>
<td>1250</td>
</tr>
<tr>
<td>3</td>
<td>1:20</td>
<td>39.8</td>
<td>84</td>
<td>10</td>
<td>1:200</td>
<td>40.8</td>
<td>760</td>
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<tr>
<td>4</td>
<td>1:20</td>
<td>37.5</td>
<td>102</td>
<td>10</td>
<td>1:200</td>
<td>38.6</td>
<td>930</td>
</tr>
<tr>
<td>5</td>
<td>1:20</td>
<td>40.2</td>
<td>79</td>
<td>10</td>
<td>1:200</td>
<td>39.8</td>
<td>840</td>
</tr>
<tr>
<td>Pooled normal serum (24 hours old)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1:20</td>
<td>45.8</td>
<td>52</td>
<td>10</td>
<td>1:100</td>
<td>51.0</td>
<td>175</td>
</tr>
<tr>
<td>2</td>
<td>1:20</td>
<td>48.0</td>
<td>43</td>
<td>10</td>
<td>1:100</td>
<td>54.9</td>
<td>135</td>
</tr>
<tr>
<td>Partially purified fraction (Ppt Ac-VI)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1:20</td>
<td>45.2</td>
<td>54</td>
<td>0.2</td>
<td>1:200</td>
<td>44.0</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>1:20</td>
<td>52.8</td>
<td>31</td>
<td>0.2</td>
<td>1:100</td>
<td>43.0</td>
<td>330</td>
</tr>
<tr>
<td>Saline</td>
<td>—</td>
<td>127</td>
<td>0</td>
<td>0.2</td>
<td>1:100</td>
<td>127</td>
<td>0</td>
</tr>
</tbody>
</table>

* Std. = standard; corrected for dilution.
† Units per ml. used for activation before dilution for assay.

METHODS

Plasma Prothrombin

Both one-stage and two-stage assays were used for the determination of plasma prothrombin. The one-stage assay used was that of Ware and Stragnell. Two-stage determinations were done by the method of Ware and Seegers.

Ac-globulin

Ac-globulin was determined using the one-stage procedure of Lewis and Ware.

Fractionation

The procedures used to obtain plasma fractions containing partially purified Ac-globulin or prothrombin were those described by Lewis and Ware.

Mechanism of Action of Ac-Globulin

Conversion to Active Serum Ac-globulin in the Presence of Thrombin

Under the influence of thrombin, Ac-G is converted to a much more active form. This activation takes place when thrombin is added to plasma, plasma fractions, or, to a lesser degree, to serum (table 1).

Fresh normal citrated plasma (9 parts of venous blood mixed with 1 part of 0.109 M sodium citrate) was clotted by adding thrombin* which had been freed of accelerator or prothrombin by adsorption with barium sulfate (50 mg. per ml.) in the presence of 0.02 M oxalate. To 0.5 ml. plasma were added 0.4 ml. saline, followed by 0.1 ml. of adsorbed thrombin (100 Iowa units per ml.). The fibrin clot was allowed to form at room temperature and then removed by winding out

* Parke-Davis bovine thrombin or purified human thrombin had the same effect.
on an applicator stick. Exactly 10 minutes after the addition of thrombin, the converted plasma was diluted 1:100 with saline (equivalent to a 1:200 dilution of the original plasma) and assayed immediately for Ac-G. As shown in table 1, conversion with thrombin resulted in about a ten-fold increase in apparent activity, regardless of the Ac-G level in the starting plasma.

When blood is allowed to clot spontaneously at room temperature, apparently not all of the plasma Ac-G is converted to the active form. When pooled normal serum thus obtained was aged overnight in the refrigerator and treated with thrombin exactly as described for plasma, a three-fold increase in apparent activity resulted (table 1).

Partially purified plasma fractions containing Ac-G can also be activated by the addition of purified thrombin (either bovine or human). Thrombin, in an amount too small to affect the assay system by itself, caused a ten-fold increase in the Ac-G activity of partially purified plasma fractions (table 1).

**Inactivation of Serum Ac-globulin**

When citrated plasma has been converted by thrombin in the manner described above, the serum Ac-G is unstable; over half of the activity disappears in an hour, and more than two-thirds in ninety minutes (fig. 1). However, if the converted plasma is diluted at least 1:100 10 minutes after adding the thrombin, stability is increased, and only about one third of the activity is lost in ninety minutes. When the partially purified fractions are converted with minimal amounts of thrombin, the stability of the activated Ac-G is even more strikingly enhanced, full activity being maintained for 2 hours. In some preparations, up to half of the active Ac-G level is still present after eighteen hours.

This increased stability suggested that the action of an inhibitor might be responsible for the rapid loss of activity in the converted plasma samples which were not diluted after conversion. In the partially purified fractions, the increased stability of the converted Ac-G was noted in precipitate Ac-III obtained by acid precipitation; hence it might be assumed that separation from the
Fig. 2.—Inactivation of active Ac-G in serum by supernatant Ac-III from acid precipitate.

Fig. 3.—Effect of adsorption with barium sulfate (50 mg./cc. plasma) on Ac-G levels in plasma, before and after conversion with thrombin. Results as per cent of normal plasma Ac-G.

Inhibitor occurred at this step. To test this assumption, supernatant Ac-III was neutralized, dried from the frozen state, and restored to the volume of the original plasma. Preliminary experiments indicate that this fraction has inhibitory properties when mixed with purified plasma fractions containing activated Ac-G.

When blood is allowed to clot, the initial rise in Ac-G activity is followed by a profound drop in activity to a low level which remains relatively constant and stable. However, if such aged serum is mixed with the reconstituted supernatant Ac-III described above, the activity drops much more rapidly than when the serum is mixed with saline (fig. 2).

These data suggest that the capacity of the inhibitor to destroy serum Ac-G is limited, thus allowing some of the active accelerator to remain in aged serum.

**Relation of the Ac-Globulin Complex to the Other Clotting Factors**

**Adsorbability of Human Ac-globulin**

Plasma Ac-G is not adsorbed appreciably on barium citrate from citrated plasma, nor on barium sulfate from oxalated plasma. The plasma Ac-G remaining
after treatment of plasma with barium citrate or barium sulfate can be converted to the active serum Ac-G by thrombin (fig. 3).

However, after treatment of plasma with adsorbed bovine thrombin, the serum Ac-G thus formed can be quantitatively removed from the converted plasma by adsorption on barium sulfate in the presence of oxalate (fig. 3). Moreover, the serum Ac-G can be partially eluted from barium sulfate with 5 per cent sodium citrate.

Therefore, although human plasma Ac-G is not adsorbed on barium sulfate or barium citrate, human serum Ac-G seems to have the same adsorption characteristics reported for SPCA.⁵

Production of Serum Ac-globulin from Aged Plasma

When fresh blood of normal Ac-G content is allowed to clot spontaneously, an appreciable amount of Ac-G activity remains in the serum after twenty-four hours (table 1) and this appears to be relatively stable. However, when oxalated plasma is aged, the Ac-G disappears almost completely and there is no appreciable increase in Ac-G activity after the addition of thrombin (table 2). Such aged plasma also fails to yield any accelerator detectable by the SPCA assay described by de Vries, Alexander, and Goldstein⁵ when clotted either by recalcification or by treatment with thrombin.

Relation to the Prothrombic Hyperactivity of Stored Plasma

A number of investigators have observed that in aged plasma there is an increase in apparent prothrombic activity by one-stage technics where Ac-G is supplied in optimal quantities.⁶⁻⁸ Ware and Stragnell⁶ found that this hyperactivity develops to a mild degree at 25 C. and that it can be largely prevented by the use of a small amount of heparin in the anticoagulant. We have found that heparin also prevents the appearance of hyperactivity at 4 C. Since Ac-G has been shown to deteriorate much more rapidly in such a heparin-oxalate mixture,⁴ it was originally thought that the effect of this anticoagulant in preventing the formation of prothrombic hyperactivity might be related to the disappearance of Ac-G. If this were true, it might be assumed that the prothrombic hyperactivity was due to gradual formation of serum Ac-G which is prevented by heparin. However, all available data indicate that such is not the case.

| TABLE 2.—Deterioration of Accelerator Globulin in Aged Oxalated Plasma Showing Prothrombic Hyperactivity |
|---------------------------------------------------------------|---------------------------------------------------------------|------------------|------------------|
| Ac-G activity                                      Prothrombin                                      | % of Std.                                      | % of normal plasma |
| Seconds | % of Std. | % of normal plasma |
| Aged oxalated plasma ........................................ 105  5  240 |
| Aged oxalated plasma converted with thrombin .......... 102  5  — |
| Saline control ........................................ 117  0  — |

* Modified one-stage prothrombin test.
When prothrombin and Ac-G levels were determined on stored refrigerated plasma over a two to three-week period, the prothrombic activity of oxalated plasma rose to 300% of its original value (measured by the modified one-stage procedure), while the Ac-G activity dropped steadily almost to zero (fig. 4).

A mixture of equal parts of normal plasma and freshly activated serum Ac-G gave the same one-stage prothrombin assay as the normal plasma mixed with saline, indicating that the prothrombin assay system is not sensitive to active Ac-G.

The Ac-G assay system has been repeatedly shown to be sensitive to small amounts of accelerator activity in both plasma and serum. However, aged plasma which showed 200 to 300% prothrombic activity showed little or no Ac-G activity, either before or after treatment with thrombin (table 2).

Since the aged, hyperactive plasma was almost devoid of Ac-G, it was thought that perhaps the addition of fresh plasma as a source of Ac-G might make it possible to demonstrate an accelerator effect as described for SPCA. Accordingly, mixtures of beef plasma with either aged hyperactive plasma or fresh plasma were tested for accelerator activity in the Ac-G assay system. In each case, the activity of the mixture was almost exactly equal to the sum of the components (table 3). Furthermore, the hyperactive plasma mixed with fresh human plasma in various proportions showed no significant accelerator effect by the modified one-stage prothrombin test.

This failure to demonstrate accelerator activity in plasma which showed a marked degree of prothrombic hyperactivity suggested the possibility that the hyperactivity observed in the modified one-stage prothrombin assay might actu-
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TABLE 3.—Accelerator Globulin Levels in Mixtures of Standard Beef Plasma and Fresh Plasma or Aged Plasma Showing Prothrombic Hyperactivity

<table>
<thead>
<tr>
<th>Prothrombin</th>
<th>Volume</th>
<th>Modified one-stage % of std.</th>
<th>Yield*</th>
<th>Units/cc.</th>
<th>Two-stage N</th>
<th>Ac-G (of standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasma</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>169</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Starting plasma</td>
<td>60 cc.</td>
<td>300</td>
<td>96%</td>
<td>195</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ppt Pr-II</td>
<td>30 cc.</td>
<td>575</td>
<td>100%</td>
<td>424</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>Ppt Pr-III</td>
<td>5 cc.</td>
<td>90</td>
<td>3%</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ppt Pr-IV</td>
<td>6 cc.</td>
<td>1840</td>
<td>61%</td>
<td>1500</td>
<td>77%</td>
<td>0</td>
</tr>
</tbody>
</table>

* Aged plasma showing prothrombic activity = 228 per cent of normal (modified one-stage prothrombin assay).

TABLE 4.—Preparation of Prothrombin from Hyperactive Aged Plasma

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Prothrombin</th>
<th>Ac-G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasma</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Starting plasma</td>
<td>60 cc.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ppt Pr-II</td>
<td>30 cc.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ppt Pr-III</td>
<td>5 cc.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ppt Pr-IV</td>
<td>6 cc.</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Yield calculated as per cent of “prothrombic activity” measured in starting plasma.

The hyperactivity was due to some change in the prothrombin molecule itself. A change in the ease of convertibility of prothrombin would not affect either the two-stage prothrombin assay (which is a measure primarily of the amount of prothrombin present), or the Ac-G assay, which is not affected by prothrombin in the test material as high as several hundred per cent of that in normal plasma. Provided that the alteration in convertibility did not involve a gross change in the other physicochemical properties of prothrombin, it should be possible to isolate this hyperactive form from aged citrated plasma which had developed prothrombic hyperactivity.

To test this assumption, purified prothrombin was prepared according to the previously described technic using as a starting material aged citrated plasma having a prothrombic activity of 300 per cent by the modified one-stage assay. Yields throughout the isolation procedure were checked by both one-stage and two-stage prothrombin assays. The results (table 4) show that the hyperactivity parallels the prothrombin content at each stage of the procedure. Furthermore, the total yield, expressed as per cent of prothrombin measured in the starting plasma, is approximately the same whether assayed by the two-stage or the modified one-stage method. The fact that the hyperactivity parallels the prothrombin concentration at every step during the purification process makes it very improbable that a factor separate from prothrombin is responsible for the hyperactivity.
DISCUSSION

In any discussion of the relation of Ac-G to other reported prothrombin conversion accelerators, the widely different assay systems used must be taken into account. In the modified one-stage prothrombin method of Ware and Stragnell, both the amount of prothrombin in the test plasma and its degree of convertibility may affect the observed clotting time. Therefore, unless the recommended heparin-containing anticoagulant is used, the test may be affected by prothrombic hyperactivity. This test is not, however, affected by an excess or dearth of Ac-G in the test plasma.

The one-stage Ac-G assay described by Lewis and Ware is unaffected by large changes in the prothrombin content of the test plasma. It measures the accelerating effect of Ac-G on the conversion of a fixed amount of prothrombin, and the observed clotting times presumably include a slight latent period required for the conversion of the inactive plasma Ac-G to the active serum Ac-G. The assay is affected by small amounts of the active accelerator which apparently speeds clotting by reducing the time of the latent period. This phenomenon was observed by Ware and Seegers using the two-stage Ac-G assay with beef plasma and serum.

Murphy, Ware, and Seegers, using the two-stage assay, concluded that Ac-G completely disappears from human serum in a few hours. This conclusion is apparently erroneous since it has been shown in this study that Ac-G exists in aged human serum in significant quantities in both the active and precursor forms. The reason for this discrepancy is obscure but it seems likely that the complexity of the two-stage assay coupled with the use of bovine reagents to measure human Ac-G could explain the difference. The prothrombin activation curves using the two-stage method were always atypical when human serum was used as a source of Ac-G and quantitation was made very difficult.

No comparisons of the Ac-G assay and the cothromboplastin assay of Mann have been attempted. However, in our hands, the assay for SPCA activity described by de Vries, Alexander, and Goldstein was significantly affected both by prothrombic hyperactivity and by traces of serum Ac-G and may, therefore, measure a combination of factors.

Investigations of Ac-G and convertin or SPCA have been complicated not only by the different assay systems used, but also by the fact that most comparative studies have attempted to correlate beef Ac-G and human SPCA. The resultant confusion regarding stability, barium sulfate adsorbability, etc., may be partially explained on this basis. Further, the apparent difference in stability between SPCA and human serum Ac-G may be related to the action of an inhibitor which rapidly inactivates most of the active serum Ac-G. Indeed, upon re-evaluation, much of the previously published data may be interpreted as supporting this concept.

Only human material was studied during the course of these investigations, and no attempt has been made to explore the differences or similarities between human and bovine Ac-G. However, on the basis of previously published work dealing exclusively with bovine systems, certain species differences are immediately apparent. The adsorbability of human serum Ac-G on barium sulfate
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is in direct contrast to the nonadsorbability on barium salts reported for bovine Ac-G. Bovine Ac-G is much more stable than human, in both plasma and serum. Experience with the bovine plasma and serum has shown, however, that dilution tends to result in a rather rapid drop in activity, whereas in human serum, Ac-G is often more stable in high dilution.

The experimental data presented here are in accord with the idea that prothrombin may exist in a hyperactive state. This is in agreement with the early proposals of Bordet and Howell, and the more recent hypotheses of Quick.

The studies of Banfi, Tanturi, and Bay indicated that the hyperactivity in stored plasma was due to a change in the prothrombin molecule. Ovren denied that this hyperactivity was due to a change in prothrombin although he provided no experimental proof to the contrary. Alexander and Landwehr provided evidence to suggest that this phenomenon was due to the evolution of a prothrombin conversion accelerator. They isolated a fraction from hyperactive plasma which was low in prothrombin but which was capable of enhancing the prothrombin activity of fresh plasma. The conclusions drawn from this experiment did not, however, take into account the fact that their assay system is very sensitive to small amounts of thrombin. A trace of thrombin is commonly found in these isolated fractions. After mixing the fraction with the normal plasma, serum Ac-G was probably generated which accounted for the accelerator effect. We were unable to detect any significant accelerator effect in the hyperactive plasma.

The prevention of prothrombic hyperactivity with small amounts of heparin is of considerable interest. Howell suggested years ago that an inert type of prothrombin exists in combination with heparin. The nature of the carbohydrate in prothrombin is obscure but there is no reason to suspect a dissimilarity to heparin. It appears that this subject warrants reinvestigation.

Quick has claimed that the difference in prothrombin time between human and dog plasma is related to prothrombin reactivity. The prothrombin time in the dog of about 6 seconds is interpreted to be the result of a very reactive type of prothrombin. However, when the modified prothrombin test of Ware and Stragnell was used, dog plasmas yielded prothrombin levels only slightly higher than human. Rabbit plasma, which also gives a 6 second Quick prothrombin time, yielded prothrombin levels almost exactly the same as human plasma. Therefore, it appears that the cause of the 6 second Quick-prothrombin time in rabbit and dog plasmas is not the result of prothrombic hyperactivity. It is probably the result of the high Ac-G levels, which are 10 to 20 times more than the Ac-G in human plasma.

The nature of the alteration in the prothrombin molecule remains obscure, but it would seem possible that this change in reactivity might have been interpreted by other investigators as indicating the existence of a second prothrombin conversion accelerator.

Conclusions

1. The results of this study support the view that there is only one accelerator of prothrombin conversion in human plasma.
2. This factor, accelerator globulin (Ac-G) exists in human plasma in a rela-
tively inert form; it is converted to the much more active serum Ac-G by very small amounts of thrombin. Not all of the plasma Ac-G is converted during the process of spontaneous coagulation. Most of the active Ac-G in serum is rapidly inactivated by an inhibitor but a small amount of residual activity remains for long periods.

3. The active human serum Ac-G can be effectively adsorbed on barium sulfate and can be eluted with citrate; the inactive human plasma Ac-G is not appreciably adsorbed on barium sulfate or on barium citrate.

4. Oxalated or citrated plasma stored at refrigerator temperature exhibits hyperactivity in modified one-stage prothrombin assays. The formation of this hyperactivity can be prevented with small amounts of heparin. It appears to be due to some change in the convertibility of the prothrombin molecule, rather than to any alteration in accelerator activity.

**Summary in Interlingua**

Le presente investigation esseva interprindite pro elucidar le mechannismo del action del globulinna accelerator in humanos.

Le resultatos dcl investigation supporta be conceptionn que le plasma del hominie contine non duo sed solo un accelerator del conversion de prothrombinia.

Iste factor G-Ac existe in le plasma human in un forma relativamente inerte. Per minusucle quantitates de thrombina illo es convertite a in G-Ac de sero le qual es muito plus active. Durante le processo de coagulation spontanee, non tone le G-Ac de plasma es convertite. Le major parte de G-Ac active in le sero es rapidemente inactivate per un inhibitor, sed un parve residuo de activitate re-manec durante un longe periodo.

Le active G-Ac de sero del homine pote esser efficacemenite adsorbite super sulfato de barium e pote esser eluite per citrato. Le inactive G-Ac de sero del homine non es adsorbite sensibilemente super sulfato de barium o citrato de barium.

Quando plasma a oxalato o citrato es magazinate a temperaturas de refrigeration, illo exhibi hyperactivitate in essayos a prothrombina secundo le ameliorate methodo Ware-Stragnell a stadio unic. Le formation de iste hyperactivitate pote esser prevenite per medio de parve quantitates de heparina. Su causa pare esser alicun cambiamento in le convertibilitate del molecula de prothrombina plus tosto que qualunque alteration del activitate accelerator.

**References**


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