Contact activation in the extrinsic blood clotting system

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Contact Activation in the Extrinsic Blood Clotting System

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Two reaction sequences of blood coagulation are currently recognized. The *intrinsic pathway* comprises factors XII, XI, IX, VIII, X, V, II, and I; the *extrinsic pathway* shares factors X, V, II, and I with the intrinsic, and also includes factor VII. The intrinsic pathway is thought to be triggered by contact of factor XII with a wettable surface, whereas the reactions of the extrinsic pathway are initiated by the action of "tissue factor" on factor VII.

During an investigation into the kinetics of the extrinsic system as measured by the thrombotest method, contact with a wettable surface was found to shorten the clotting times in this system (1). This phenomenon has already been described (2), but since agreement has not been reached concerning the responsible mechanism, it seemed worthwhile to investigate this particular point further.

Materials and Methods

All plasmas (normal as well as congenitally deficient) were prepared contact-free, centrifuged for 30 min at 30,000 g, and then stored at $-20°$ C in plastic tubes.

Factor V-poor plasma was prepared by storing normal oxalated plasma at 37° C. The clotting-factor contents of this plasma were; factor II: 75%; factor VII: 62%; factor X: 50%; factor V <1%.

Ba-stearate-absorbed plasma is normal citrate plasma treated with 50 mg/ml Ba-stearate (K and K laboratories Inc., Plain View, N. Y., U.S.A.; lot 45884 L) for 10 min at 37° C, and centrifuged for 10 min at 30,000 g. This plasma contained: factor II: 80%; factor VII: 105%; factor V: 8%; factor XII: 100%, and no measurable amount of factor XI (3).

Exhausted plasma was prepared according to Nossel (4) by admixture of 30 mg/ml of celite (Hyflo Super-cel, Johns-Manville, N.Y., U.S.A.) before centrifugation, *or* by treatment with 5 mg/ml of celite only.

PTA-deficient plasma was a gift from Dr. S. I. de Vries, Wilhelminagasthuis, Amsterdam (5). Improved PTA-deficient plasma was prepared from this plasma according to Soulier and Prou Wartelle (6).

Contact product (C.P.) was prepared according to Niewiarowski (7) using celite as absorbent. The preparation contained traces of factor II (3%), factor VII (6%), and factor X (3%). An activity of 100% of this preparation was defined as the activity that, when twice diluted, caused the same clotting time as a suspension of celite (10 mg/ml) in the following reaction mixture: normal plasma 0.2 ml; phospholipid suspension 0.1 ml; C.P. *or* celite suspension 0.1 ml; $CaCl_2$ 25 mM 0.1 ml.

Heated C.P. was prepared by heating the C.P. for 30 min at 65° C in a water bath.

All other materials and methods were as previously described (1).

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Experimental Results

The basic test of this investigation consisted of incubation at 37° C of a plasma diluted 1 in 10 with buffer, with or without various admixtures. At given times a 0.05 aliquot of this plasma was subsampled into 0.25 ml thrombotest reagent. The clotting time thus obtained was plotted against the incubation time. To describe the activation numerically, the clotting time (a) at zero incubation time and the clotting time (b) after an arbitrary incubation time (c) are indicated by the notation $t_{o\text{-}c}$ (a; b). Thus $t_{0\text{-}2}$ (80; 69) means that in a given experiment the thrombotest time at zero time was 80 seconds and after two hours 69 seconds. The values called zero-time values were obtained as quickly as possible after mixing in the kaolin (between 10 and 30 sec). Fig. 1 and 2 and Table 1 show that a concentration of 0.156 mg kaolin per ml incubation mixture ensures maximal activation of the thrombotest time and minimal absorb-

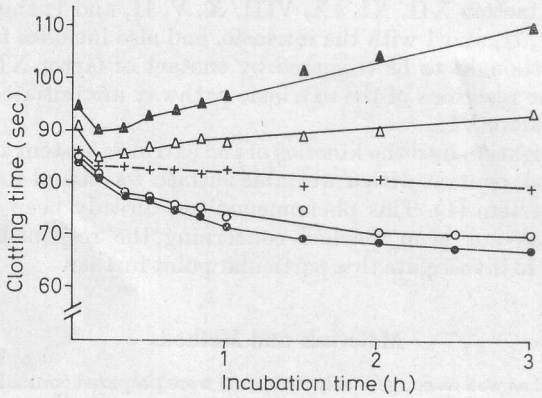

Fig. 1. The activation of the thrombotest reaction in normal plasma (1 : 10 dilution) after incubation with various concentrations of kaolin. ▲ 5 mg/ml; △3.75 mg/ml; + 0.05 mg/ml; ○ 0.10 mg/ml; ● 0.156 mg/ml.

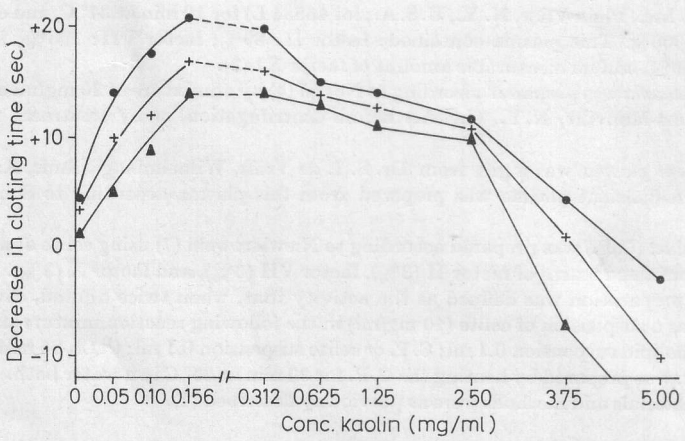

Fig. 2. The activation of the thrombotest reaction in normal plasma (1 : 10 dilution) by incubation with kaolin. ▲ after 1 hr; + after 2 hrs; ● after 4 hrs.

Table 1. The Effect of Incubation with Kaolin on the Clotting Factors of Normal Plasma.

Conc. of Kaolin (mg/ml)	Incubation time (min)	F. II	F. V	F. VII	F. X
0.	240	8.4	5.8	9.8	8.4
0.156	240	7.5	6.0	11.5	7.4
0.312	240	6.5	3.2	10.2	5.8
0.625	90	5.8	1.7	9.6	5.6
1.250	120	6.1	1.7	7.8	6.4
2.500	180	6.4	.25	5.8	4.2
3.750	240	4.2	—	3.6	1.6
5.000	300	3.4	.02	2.3	.7

One in 10 diluted normal plasma was incubated with kaolin. After the incubation time the plasma was centrifuged for 10 min at 20.000 g. The clotting factor concentration was determined and expressed in % of the same normal pool plasma the experimental sample originated from.

ance of clotting factors. Consequently, this concentration was used in further experiments. At this concentration of kaolin it made no difference whether the sample was incubated in a glass or a plastic tube. Even at a lower kaolin concentration (0.10 mg/ml) no differences were found, as can be seen from the following figures: in glass, $t_{0\text{-}1}$ (83; 79); $t_{0\text{-}2}$ (83; 78); $t_{0\text{-}3}$ (83; 71); in plastic, $t_{0\text{-}1}$ (83; 78); $t_{0\text{-}2}$ (83; 76); $t_{0\text{-}3}$ (83; 71); (means of sixfold estimations).

To determine the pathway of contact activation in the extrinsic system, the activation of various deficient plasmas was investigated. For plasmas deficient in factors, II, VII, and X, the test procedure had to be modified because of the sensitivity of the thrombotest reaction for these factors. In these cases the 1 in 10 dilution of deficient plasma was incubated with kaolin, but the test was carried out by subsampling into 0.5 ml thrombotest, together with 0.05 ml of a 1 in 10 dilution of fresh normal, non-contacted plasma.

The results are shown in Table 2, from which it can be seen that factor XII and factor VII are absolutely necessary for activation of the extrinsic system, whereas

Table 2. Contact Activation of the Extrinsic System in Deficient Plasmas.

Plasma	t_0	$t_{1/2}$	t_1	t_2	t_4	t_0–t_4
F. XII deficient	83	83	82	82	79	4
F. XI deficient	101	98	95	92	90	11
F. X deficient	97	91	89	84	79	18
F. IX deficient	97	87	83	78	72	25
F. VIII deficient	89	82	77	72	66	23
F. VII deficient	96	97	95	94	93	3
F. V deficient	109	104	98	94	92	17
F. II deficient	93	89	82	78	76	17
Normal plasma	86	77	72	69	65	21
Normal plasma + 3 mM EDTA	83	76	73	67	63	20

The tests were carried out as described in the text. The subscript of t denotes the incubation time. The final concentration of kaolin was 0.156 mg/ml. The figures are derived from triplicate experiments, where the thrombotest time of the plasma under investigation was measured every 10 min.

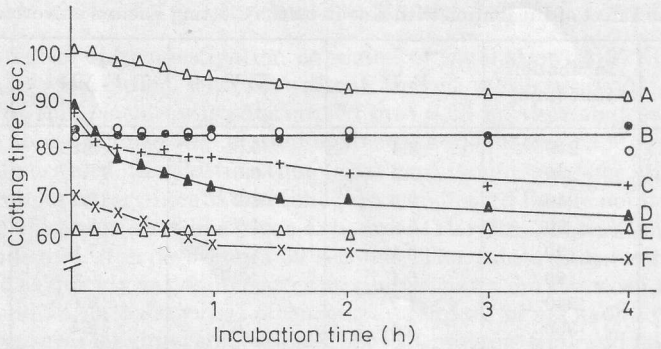

Fig. 3. The activation of the thrombotest reaction by incubation with 0.156 mg/ml kaolin in various plasmas. A Factor XI-deficient plasma; B Exhausted and non-exhausted factor XII-deficient plasma; C Improved factor XI-deficient plasma; D Normal plasma; E Exhausted normal plasma; F Ba-stearate-absorbed plasma.

factors X, IX, VIII, V, and II are not. The results of the experiment with factor XI-deficient plasma indicate that activation of VII is possible without factor XI, but that the presence of factor XI enhances the activation. This is emphasized by Fig. 3, which shows not only that congenitally factor XI-deficient plasma is capable of activation, but also that after absorption with celite (5 mg/ml) to remove possible traces of factor XI, this capability was still present. Ba-stearate-absorbed plasma, which contained no measurable amounts of factor XI, was capable of activation in the extrinsic system. Ba-stearate, it should be noted, seems to activate plasma to a certain degree. The process that brings about "exhaustion" of normal plasma (10 min incubation at 37° C in the presence of 30 mg/ml celite) apparently causes maximal activation of the extrinsic system, but it has no effect on factor XII-deficient plasma. The contact product (C.P.) prepared according to Niewiarowski (7) can cause activation of factor XII-deficient plasma in the absence of kaolin (Fig. 4). When heated for 30 min at 65° C, the C. P. lost its activity either to activate the exhausted Hageman plasma

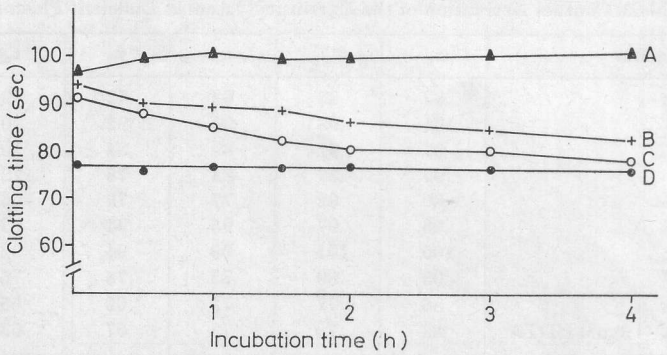

Fig. 4. The activation of the thrombotest reaction by addition of contact product in various plasmas. A Exhausted factor XII-deficient plasma with added C.P., after heating at 65° C for 30 min; B Exhausted factor XII-deficient plasma; C Factor XII-deficient plasma; D Exhausted normal plasma.

(Fig. 4) to correct a factor XII- or factor XI-deficient plasma. The degree of activation was quantitatively related to the amount of factor XII and the amount of C. P. (Fig. 5). The variation of factor XII was brought about by mixing normal and factor XII-deficient plasma. C. P. was added to exhausted factor XII-deficient plasma in the other series of experiments.

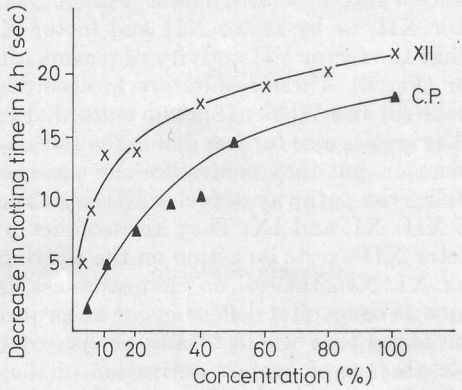

Fig. 5. The influence of the concentration of factor XII and contact product on the activation of factor XII-deficient plasma.

The extent of activation was also dependent upon the amount of factor VII present. It was not possible to express this in a simple relationship, as was done for factor XII and C. P. Consequently, Fig. 6 shows the relationship between the amount of factor VII known to be present at zero time and the amount apparently present after 4 hrs of activation by kaolin.

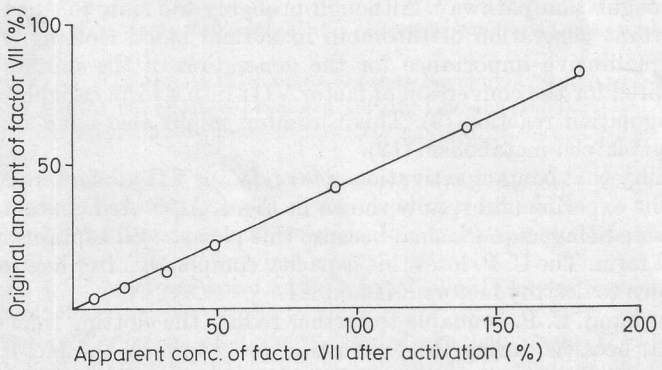

Fig. 6. The influence of activation on the apparent amount of factor VII in mixtures of normal and factor VII-deficient plasmas.

Incubation with neutralized thorium chloride did not prevent activation, as can be seen from the following figures: with ThCl: $t_{0\text{-}3}$ (85; 74); without ThCl: $t_{0\text{-}3}$ (84; 72); (mean of 6 estimations).

Discussion

The experimental results indicate that contact, as produced by glass or kaolin, shortens the clotting time in a thrombotest system by a mechanism dependent upon factor XII and factor VII. The other clotting factors are not compulsatory for this phenomenon, although factor XI has a clear-cut accelerating effect. This suggests that factor VII can be converted into activated factor VII (F. VII) not only by tissue factor but also by factor XII, or by factor XII and factor XI together. This also appears from the fact that the factor VII activity of plasma after contact activation was found to be higher (Fig. 6). These results are in accordance with the view of Soulier and Prou-Wartelle (6) and Waaler (8), who state that factor XII is compulsatory for this process. The results also further define the role of factor XI as that of a non-compulsatory accelerator, but they contradict the conclusion of Shanberge and Matsuoka (2), who visualize the pathway of factor VII activation by foreign surfaces as mediated by factors XII, XI, and IX. They also conflict with the intuitive idea that in all likelihood factor XII exerts its action on the clotting mechanism via or in combination with factor XI. Nonetheless, no circumstances known to promote the absence of factor XI, such as congenital deficiency, or absorption with Ba-stearate of normal plasma could be found that would totally abolish contact activation of the system tested. Complete absence of contact activation of the extrinsic system was found only in the absence of contact, in the absence of factor XII, or in the absence of factor VII. Yet factor XI does play a role in factor VII activation in normal blood, since its presence markedly enhances the contact activation observed. Therefore, both the following reaction schemes seem possible:

$$\text{(a)}\quad \text{XII} \xrightarrow{\text{contact}} \text{XII}_a \qquad \text{VII} \xrightarrow{\text{XII}_a} \text{VII}_a$$

$$\text{(b)}\quad \text{XII} \xrightarrow{\text{contact}} \text{XII}_a \qquad \text{XI} \xrightarrow{\text{XII}_a} \text{XI}_a \qquad \text{VII} \xrightarrow{\text{XI}_a} \text{VII}_a$$

It is interesting to note that these reaction sequences constitute a short-circuit of the intrinsic coagulation pathway. Although probably too slow to exert any quantitatively important generation of thrombin in normal blood clotting, this pathway might be of qualitative importance for the generation of the minute amounts of thrombin required for the conversion of factor VIII into a form capable of participating in the coagulation reaction (9). This thrombin might also have an influence on factor V and on platelet metabolism (12).

The probability that contact activation acts on factor VII via factors XII and XI is increased by the experimental results shown in Fig. 4. Activated contact product can activate exhausted Hageman Plasma, because this plasma still contains its factor VII in unactivated form. The C. P. loses this capacity completely after heating to 65° C, a procedure known to destroy factors XII and XI.

On the other hand, C. P. is unable to further reduce the clotting time of exhausted normal plasma, because during the exhaustion procedure all the factor VII in that plasma has already been converted into its active form. Ca^{++} did not appear to be necessary for the activation, since 3 mM EDTA had no inhibitory effect (Table 2).

We are unable to put forward a hypothesis to explain the difference between the findings of Shanberge & Matsuoka and our own experiments with respect to the role of factor IX in the activation of factor VII. The possibility must be kept in mind that another factor (Tatsumi factor ?) plays a role in the process. Thorium chloride or thorium hydroxide did not inhibit the activation.

Summary

After contact activation, factor XII can convert factor VII into its active form. Factor XI enhances this action but appears not to be a compulsatory part of the system. The short circuit of the intrinsic system constituted by this pathway may be important in triggering the intrinsic coagulation system.

Résumé

Après activation par contact, le facteur XII peut convertir le facteur VII en sa forme active. Le facteur XI augmente cette action mais n'apparaît pas indispensable. Le court circuit du système intrinsèque constitué par cette voie peut être important dans le déclenchement du système intrinsèque de la coagulation.

Zusammenfassung

Nach Kontaktaktivierung vermag Faktor XII Faktor VII in seine aktive Form umzuwandeln. Faktor XI steigert diese Wirkung, scheint aber kein unbedingt notwendiger Reaktionspartner in diesem System zu sein. Der Kurzschluß des endogenen Systems, der durch diesen Weg hergestellt wird, könnte für die Aktivierung des endogenen Gerinnungssystems von Bedeutung sein.

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