DEVELOPMENT OF INCREASED FACTOR VII ACTIVITY DURING THE SPONTANEOUS COAGULATION OF BLOOD

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During the spontaneous coagulation of normal blood, in the absence of tissue thromboplastin, Factor VII (proconvertin) activity increases 2.5 to 3 times. This is specific for Factor VII and not related to possible changes in Factor X (Stuart factor) (1). Hougie (2) made the same observation and added that Factor VII activity developed normally during clotting of blood deficient in Factor X.

The development of Factor VII activity in blood from patients with coagulation disorders was studied by Aas (3) and Hougie (2). In addition, there are conflicting reports of Factor VII assays on serum from patients with coagulation abnormalities (4–7). Such variations might be technical but could also depend upon the disorder studied. Therefore, the development of increased Factor VII activity during clotting of blood from various patients, encompassing most known coagulation disorders, has been investigated. Using a standardized technique, it was found that some clotting factors are necessary for the development of increased Factor VII activity, while others appear to be unnecessary.

MATERIALS AND METHODS

Thrombin. Bovine thrombin (Topostasin, Roche, Basel) was used. The untreated preparation contained slight Factor VII activity. Therefore, it was reconstituted to 100 NIH U per ml, adsorbed with 100 mg $BaSO_4$ per ml, and centrifuged at 40,000 rpm (144,000 × G) for 60 minutes. When 0.2 ml of this preparation, containing 10 NIH U per ml, was added to 1.8 ml normal or Factor VII deficient blood, clotting occurred within 30 seconds, and the Factor VII assay results were not altered. This concentration of thrombin was sufficient to clot an equal volume of plasma in less than 10 seconds.

Thromboplastin. Saline-extracted human brain was prepared by the method of Hjort (8).

Human proconvertin reagent. This was prepared from normal human serum by the technique of Hjort (8).

Bovine proconvertin reagent. This was an eluate of bovine serum (9). This preparation and the human proconvertin preparation (above) contain considerable amounts of Factors IX and X. The presence of these "contaminants" does not alter the interpretation of data resulting when these preparations are used (see Figure 2).

Adsorbed, Seitz-filtered ox plasma. Ox plasma, deficient in Factor VII and prothrombin, prepared according to Hjort (8), served as a potent, constant source of Factor V in the Factor VII assay.

Normal blood. Blood samples from 5 donors (laboratory personnel), without coagulation abnormalities, were used as normal.

Deficiency blood. Factor deficient blood was provided by the following patients: 5 Factor IX (PTC), 5 Factor VIII (AHF), 1 Factor VII, 1 Factor V, and 1 PTA deficient. The diagnoses were based on history, clinical examination, mixing experiments, and specific assay of the deficient factor. Clinically, all patients had severe deficiencies, with the level of the deficient factor less than 2 per cent of normal. Patients deficient in Hageman factor and Factor X were not available for study. In these instances, lyophilized serum samples were studied for Factor VII activity. Hageman trait serum was provided by Mr. Herman Rierson, Chapel Hill, N. C., and Stuart (Factor X) deficient sera by Drs. J. B. Graham, Chapel Hill, N. C., C. Hougie, Charlottesville, Va., and J. Roos, Utrecht, Holland. Patient J. P. (10) was used as the source of Factor VII substrate. This plasma was stored at − 20° C until thawed for use in the Factor VII assay.

Precollection preparation. Three series of *new* 16 × 95 mm tubes were prepared. The first series contained 0.2 ml physiological saline in each of 6 tubes, and the first tube contained, in addition, 0.2 ml of 3.1 per cent sodium citrate dihydrate. The second series had 0.2 ml of 1 : 10 thromboplastin per tube, with 0.2 ml of 3.1 per cent citrate in the first tube as well. The third series had 0.2 ml of 10 NIH U per ml thrombin in each tube, with citrate in the first tube, as in the other series.

Collection of blood. The blood was drawn into 20-ml glass syringes (comparable results were obtained with siliconized syringes), distributed quickly into prepared tubes, 1.8 ml per tube, and incubated at 37° C. The zero time was the time of addition of blood to the first tube. At the stated incubation time (5, 15, 30, 60 or

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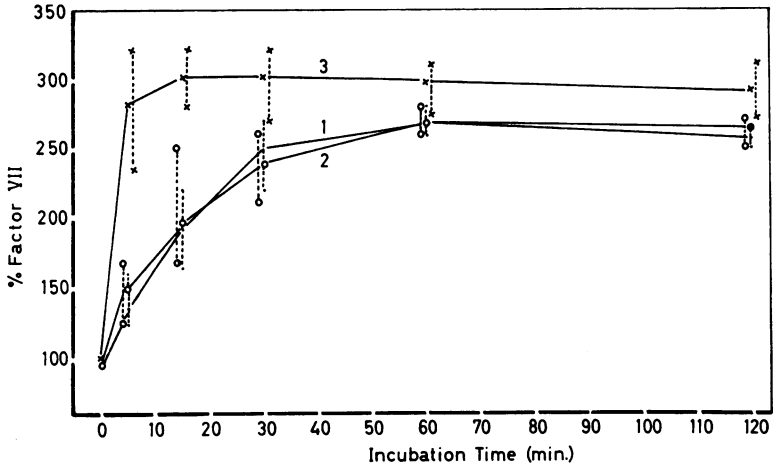

FIG. 1. NORMAL BLOOD—DEVELOPMENT OF INCREASED FACTOR VII ACTIVITY DURING CLOTTING WITH ADDED: SALINE, CURVE 1 (•———•); THROMBIN, CURVE 2 (O———O); AND THROMBOPLASTIN, CURVE 3 (×———×). Five subjects were studied and the range of the results for each of the additives is indicated by broken lines at each incubation time. The curves were constructed by connecting the points representative of the average of the determinations for each incubation time.

120 minutes), 0.2 ml of 3.1 per cent citrate was added to the appropriate tube and mixed well. For one special experiment, 0.2 ml of 30 mM EDTA was substituted for the citrate. As soon as possible after anticoagulant addition, the samples were centrifuged at 2,500 rpm (1,700 × G) for 5 minutes, diluted, and assayed in the specific Factor VII assay.

Factor VII assay. This test was similar to that previously used (1). The test sample usually was diluted 1 : 10 in Dilution Fluid II (8) and then mixed with an equal volume of adsorbed, Seitz-filtered ox plasma. One-tenth ml of this mixture (final dilution 1 : 20) was added to 0.1 ml of Factor VII deficient plasma and 0.1 ml of thromboplastin of optimal concentration. After 3 minutes at 37° C, the mixture was recalcified with 0.1 ml of 35 mM $CaCl_2$ (optimal), and the clotting time recorded. Different dilutions of normal, human plasma were assayed to construct a standard curve for conversion of clotting times to per cent. This was a straight line on a logarithmic scale.

RESULTS

1. Normal blood. Preliminary experiments, and also the work of Aas (3), suggested that Factor VII activity increases little, or not at all, during coagulation of blood with certain clotting defects. This could be the result of impaired or slow thrombin production common to nearly all such disorders. Alternatively, some defect in plasma thromboplastin formation could be responsible. The studies to be presented were designed to explore these possibilities and determine what, if any, effect preformed thrombin and tissue thromboplastin had upon the results. Therefore, each blood was incubated with three different additives: *1*) 1/10 volume of saline, *2*) 1/10 volume of thrombin (10 NIH U per ml), *3*) 1/10 volume of thromboplastin (diluted 1/10 in saline). The blood clotted after 5 and before 15 minutes of incubation in the first series, and within 30 seconds in the second and third series of tubes. The concentration of thromboplastin used produced only a slightly greater than normal increase in Factor VII activity (see Figure 1). More concentrated thromboplastin produced up to tenfold increases in Factor VII activity (3). At intervals, the reactions were stopped by citrate addition, and the Factor VII activity of that sample determined.

To confirm previous work (1), and to establish the effect of the various additives upon the development of increased Factor VII activity in normal blood, five normal samples were studied. Figure 1 shows the average and the range of these results. Curve 1, saline addition, is comparable to previous studies of normals (1, 3) and may be considered as the control. Curve 2, thrombin addition, shows that preformed thrombin did not alter the normal pattern, even though the clotting times of the samples were reduced. Curve 3,

thromboplastin addition, shows a maximum that is reached more rapidly and is slightly greater than that of curves 1 and 2.

On one normal, an additional experiment was performed in the same manner, except that citrate, plus the usual additives, was present in *all* tubes prior to the addition of blood. In each series there was only slight, gradual development of Factor VII activity, to a maximum of 130 per cent in 2 hours. Therefore, surface activation of Factor VII (11) is not directly responsible for the development of increased Factor VII activity during coagulation. However, surface reactions, mediated through the clotting mechanism, play an important role in the development of such activity (see below). These experiments further show that preformed thrombin has little influence on the activation of Factor VII.

EDTA, which is a more effective decalcifying agent than citrate (8), was used as the anticoagulant in another experiment on a normal. The results were intermediate (maximum activity, 184 per cent) between those of the above and the usual experiments with citrate (Figure 1). These findings suggest that the presence of calcium is important for the development of increased Factor VII activity.

2. Factor VII (proconvertin) deficiency. Aas (3) reported that hypoproconvertinemic blood developed negligible proconvertin activity during clotting. In the present study, Factor VII deficient blood was studied in the usual way, see Figure 2A. The clotting times of the samples were similar to those of the normal study. In no instance was there more than a minimal increase (up to 12 per cent) in Factor VII activity.

Although none has been reported, a specific inhibitor might be responsible for Factor VII deficiency. One-tenth vol of proconvertin (Factor VII) reagent was added to Factor VII deficient blood and the developed activity studied. Two preparations, one of human and one of bovine origin, were used. Each contained, compared to normal plasma, about 200 per cent of the factor. Figure 2B shows the results. Both of the preparations were activated to two to three times the original concentration during the clotting of the blood. Thrombin addition to the bovine preparation did not alter the activation. The difference between the preparations perhaps may be due to species differences. However, regardless of the preparation used, it can be seen that in Factor VII deficiency there is no lack of ability to activate the factor, if it is provided.

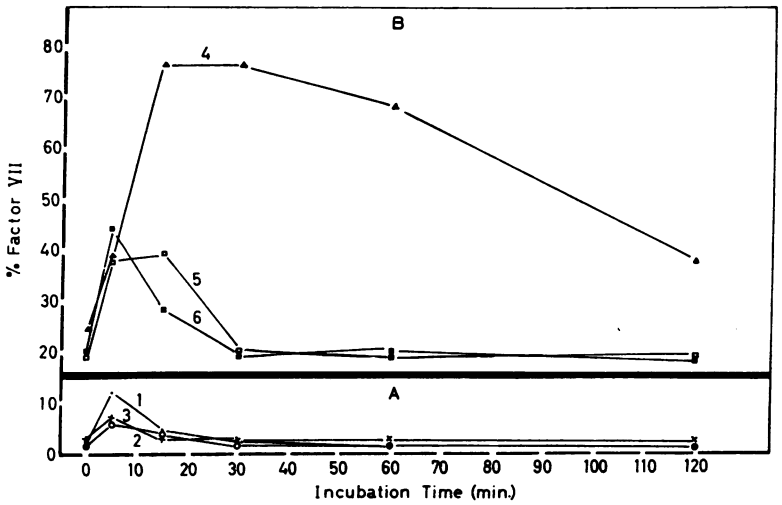

FIG. 2. FACTOR VII DEFICIENT BLOOD—DEVELOPMENT OF INCREASED FACTOR VII ACTIVITY DURING CLOTTING. **A.** Additives used: saline, curve 1 (•———•); thrombin, curve 2 (○———○); and thromboplastin, curve 3 (×———×). **B.** Additives used: human Factor VII, curve 4 (▲———▲); bovine Factor VII, curve 5 (□———□); and bovine Factor VII plus thrombin, curve 6 (■———■).

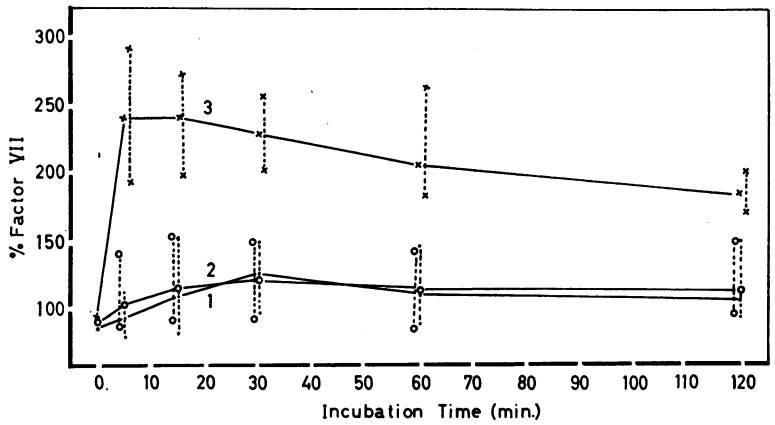

FIG. 3. FACTOR IX DEFICIENT BLOOD—DEVELOPMENT OF INCREASED FACTOR VII ACTIVITY DURING CLOTTING WITH ADDED: SALINE, CURVE 1 (·———·); THROMBIN, CURVE 2 (○———○); AND THROMBOPLASTIN, CURVE 3 (×———×). Five subjects were studied and the range of the results, for each of the additives, indicated by broken lines at each incubation time. The curves were constructed by connecting the points representative of the average of the determinations for each incubation time.

3. Factor IX (PTC) deficiency. Previous studies suggested that the development of Factor VII activity may be impaired in hemophilia (3, 6), but these did not distinguish between the two major types, Factor VIII and Factor IX deficiency. Alexander and de Vries (6) reported impaired evolution of SPCA (Factor VII) activity which could be normalized by addition of sufficient amounts of thromboplastin. Aas (3) reported similar findings.[1] White, Aggeler and Glendening (4) observed low SPCA activity in PTC deficiency and normalized the activity by thromboplastin addition.

Five patients with Factor IX deficiency were studied in a fashion identical to that of the normal. The average and the range of results are shown in Figure 3. In most of the saline samples, clotting occurred between the 15- and 30-minute samples. However, in some experiments clotting occurred between 30 and 60 minutes. With either saline or thrombin addition there was little increase in Factor VII activity, even though the plasma contained nearly normal amounts of Factor VII. The addition of thromboplastin resulted in a pattern resembling the normal (cf Figure 1, curve 3). However, the maximum attained was somewhat lower than normal.

Artificial delay of normal clotting, by incubation of the samples at 4° C, only postpones development of Factor VII activity (1); thus, it would seem that the present findings are not due to the prolonged clotting times of the patient's blood. In two of the patients, the increase in Factor VII activity was studied before and immediately after a plasma transfusion (500 ml). Following this, the marked defect in the development of increased Factor VII activity still existed, even though the clotting time of the blood was nearly normal. The maximum value reached, while slightly higher than the pretransfusion level (164 in one, and 148 per cent in the other), was still far below that seen with thromboplastin addition (Figure 3). If it were merely a question of clotting time, both the thrombin and the post-transfusion curves should have been comparable to the thromboplastin curve. Thus, a relationship exists between Factor IX and the development of increased Factor VII activity.

4. Factor VIII (AHF) deficiency. Figure 4 shows the results obtained in a study of five Factor VIII deficient patients, all with markedly prolonged saline sample clotting times. Clearly, there is defective development of increased Factor VII activity with saline or thrombin addition.

[1] In a personal communication, Aas has stated that the patient he studied is now know to be Factor IX deficient. Indeed, this same patient is included in the present investigation.

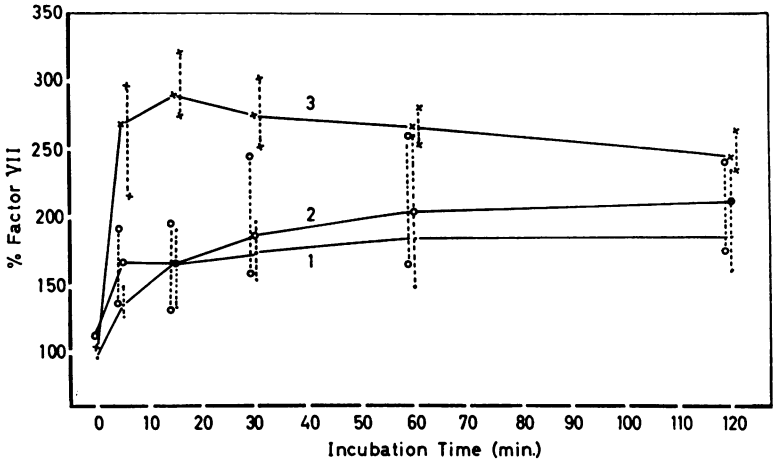

FIG. 4. FACTOR VIII DEFICIENT BLOOD—DEVELOPMENT OF INCREASED FACTOR VII ACTIVITY DURING CLOTTING WITH ADDED: SALINE, CURVE 1 (•———•); THROMBIN, CURVE 2 (○———○); AND THROMBOPLASTIN, CURVE 3 (×———×). Five subjects were studied and the range of the results, for each of the additives, indicated by broken lines at each incubation time. The curves were constructed by connecting the points representative of the average of the determinations for each incubation time.

However, the results are normal with thromboplastin addition.

One of the patients with Factor VIII deficiency was studied on two occasions. The first study was made during a period when he was transfused twice daily because of hemorrhage. These results were indistinguishable from the normal. Five days following his last transfusion, the second study showed the abnormal pattern of Factor VIII deficiency.

These studies suggest that Factor VIII, as well as Factor IX, is necessary for the normal development of increased Factor VII activity. However, the role of Factor VIII may be less important than that of Factor IX.

5. PTA deficiency. One patient with PTA deficiency was studied. The results are shown in Figure 5. There was a marked defect in the development of Factor VII activity with saline and with thrombin addition. The thromboplastin curve was normal. These data suggest that PTA, as well as the other hemophilia factors, is necessary for the development of increased Factor VII activity in the normal.

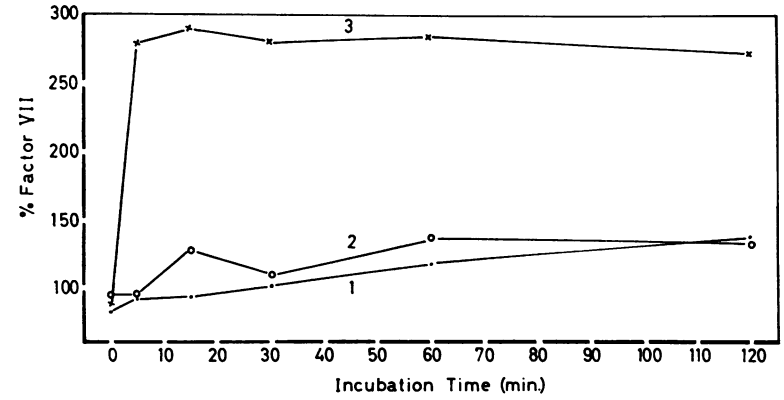

FIG. 5. PTA DEFICIENT BLOOD—DEVELOPMENT OF INCREASED FACTOR VII ACTIVITY DURING CLOTTING WITH ADDED: SALINE, CURVE 1 (•———•); THROMBIN, CURVE 2 (○———○); AND THROMBOPLASTIN, CURVE 3 (×———×).

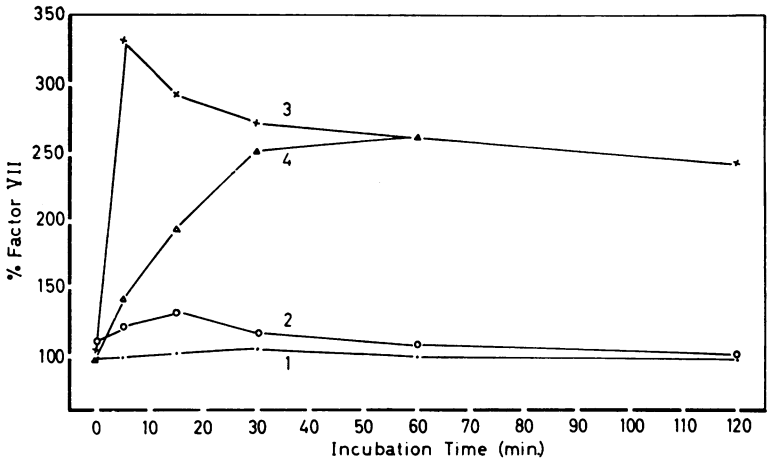

FIG. 6. NORMAL BLOOD IN NONWETTABLE TUBES—DEVELOPMENT OF INCREASED FACTOR VII ACTIVITY DURING CLOTTING OF NORMAL BLOOD WITH ADDED: SALINE, CURVE 1 (•———•); THROMBIN, CURVE 2 (○———○); THROMBOPLASTIN, CURVE 3 (×———×); AND "PURIFIED" HAGEMAN FACTOR, CURVE 4 (△———△).

6. Hageman deficiency. No patient was available for the study of Hageman factor deficiency. However, a lyophilized serum sample from a well studied Hageman trait patient (11, 12) was available. Several Factor VII determinations were performed on this sample on different occasions. The average of these results was 98 per cent, which is only one-third of the normal serum concentration. These findings suggests that Hageman factor has a role in Factor VII activation.

The relationship of Hageman factor to surface contact is well known (11–15). To study the role of Hageman factor and surface reactions in development of Factor VII activity, the original experimental design was modified to exclude surface contact during the clotting of normal blood. The blood was drawn into siliconized syringes and allowed to clot in Lusteroid tubes. Testing was carried out in the usual manner. Figure 6 shows the results. Little, if any, Factor VII ac-

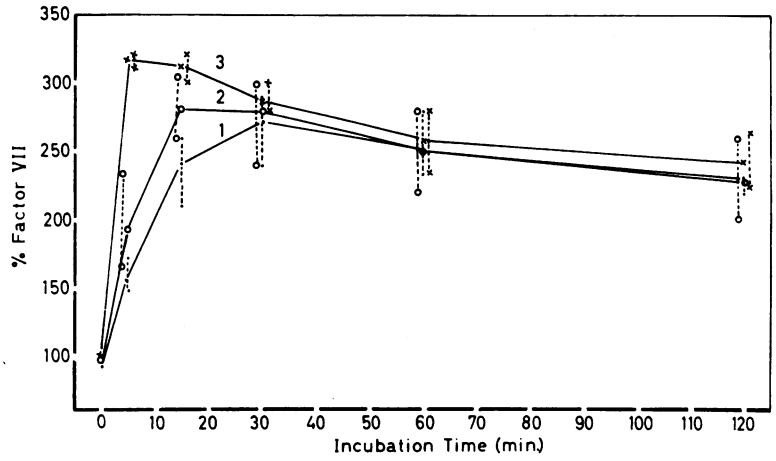

FIG. 7. THROMBOCYTOPENIC BLOOD—DEVELOPMENT OF INCREASED FACTOR VII ACTIVITY DURING CLOTTING WITH ADDED: SALINE, CURVE 1 (•———•); THROMBIN, CURVE 2 (○———○); AND THROMBOPLASTIN, CURVE 3 (×———×). Three subjects were studied and the range of the results, for each of the additives, indicated by broken lines at each incubation time. The curves were constructed by connecting the points representative of the average of the determinations for each incubation time.

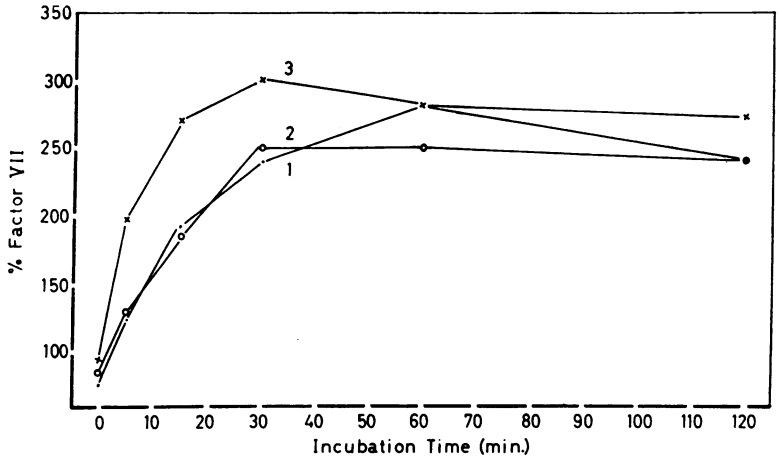

FIG. 8. FACTOR V DEFICIENT BLOOD—DEVELOPMENT OF INCREASED FACTOR VII ACTIVITY DURING CLOTTING WITH ADDED: SALINE, CURVE 1 (·———·); THROMBIN, CURVE 2 (O———O); AND THROMBOPLASTIN, CURVE 3 (X———X).

tivity developed during clotting of samples to which saline and thrombin had been added. In contrast, thromboplastin addition corrected the defect. These results are identical to those of the three types of hemophilia (Figures 3, 4 and 5).

Using this same modified technique, the effect of the addition of 0.1 vol of a partially refined Hageman factor preparation (13) was studied. This preparation is devoid of all clotting factors except Hageman and, perhaps, PTA (11). Curve 4 of Figure 6 shows that, with this addition, increased Factor VII activity developed normally (cf Figure 1). Since the blood studied was from a normal, it lacked only the "active" form of the Hageman factor. The results indicate that this activity was supplied by the preparation. Without this "active" form, i.e., in the absence of surface contact, increased Factor VII activity cannot develop.

7. Thrombocytopenia. Three patients with thrombocytopenia were investigated. Their platelet counts were 1,600, 1,600 and 9,000 per mm^3, and their clotting factors were all in the normal range. Figure 7 shows that the results were normal. The clotting times of the saline samples were within the normal range. Prothrombin consumption was not measured.

8. Factor V deficiency. Factor VII activity has been reported to be normal in Factor V deficiency (3, 7). One patient, the same one studied by Aas (3) and Owren (16), was available for study. The patient's whole blood clotting time was twice the normal average. Similarly, the clotting time of the saline samples was prolonged. Figure 8 shows that the results were normal.

9. Factor X (Stuart) deficiency. A previous study reported that Factor X was not needed for the development of increased Factor VII activity (2). Since Factor VII activity was found to be relatively stable in serum for at least several hours, lyophilized samples of Factor X deficient serum were assayed for Factor VII content. The average of at least four determinations on each of these samples was 256 per cent. This is comparable to the content of normal serum.

10. Artificial system. The data obtained from the patient material indicated that certain factors are necessary for the development of the normal increase in Factor VII activity. Therefore, an attempt was made to devise an "artificial" system to investigate the mechanism further. Aas (3), Hjort (8), and others have shown that increased activity develops in a mixture of tissue thromboplastin, proconvertin (Factor VII) and calcium. An attempt was made to mix human proconvertin reagent with "purified" factors: activation product, cephalin, Factors V, VIII, IX and X. In this fashion it was hoped to establish the role of the various factors in the activation of Factor VII. Indeed, an increased activity did develop in a complete incubation mixture. However, these mixtures were found to be thromboplastin generation systems which function normally without Factor VII (17). Thus, this attempt failed in its pri-

TABLE I

The clotting factors which are necessary and those which are not necessary for the development of increased Factor VII activity

Necessary	Unnecessary
Factor VII	Factor V
Hageman	Factor X
PTA	Platelets
Factor VIII	
Factor IX	
Calcium	

mary purpose, and the experimental details are not included.

DISCUSSION

It was confirmed that during the clotting of normal blood, Factor VII activity is increased to levels two to three times that found in plasma. Since nonspecific influences could produce such results, it first is necessary to discuss the specificity of the results. Essentially no activity develops during the coagulation of Factor VII deficient blood (Figure 2A). When a refined Factor VII preparation is added, activity similar to the normal develops (Figure 2B). Since the patient's blood lacks only Factor VII, with normal concentrations of other clotting factors, the results are explainable by the presence or absence of Factor VII.

Surface contact activation of Factor VII possibly could be responsible for the high activity in normal blood. However, the amount of glass contact was standardized for all experiments, yet differences were obtained in the results. Further, it was shown that, in the absence of coagulation, this standard contact produced only a slight increase in activity, even though the initial Factor VII concentration was normal. These data suggest that the increase in Factor VII activity is not due to direct activation by contact.

The activation of Factor VII may somehow depend upon a normal clotting time, since delayed clotting is common to nearly all the bloods with abnormal results. Acceleration of clotting by thrombin did not influence the activity. In contrast, thromboplastin addition, which also accelerated the clotting, gave a markedly different pattern, except in Factor VII deficiency. Artificial prolongation of the clotting time of normal blood, by cooling, only delays the development of Factor VII activity in glass (1). All these observations indicate that the results are not due to nonspecific influences, but are specifically dependent upon the activation of Factor VII.

Earlier studies (3, 5–7) suggest that, in certain deficiency states, the developed Factor VII (SPCA, proconvertin) activity may not be normal. The present findings show that the development of increased Factor VII activity depends upon certain of the clotting factors. Thus the clotting factors may be divided into two groups: those necessary and those unnecessary for the development of increased Factor VII activity during spontaneous coagulation of blood (Table I).

Calcium, Hageman factor, PTA, Factor VII, Factor VIII and Factor IX are necessary for such activity to develop (Figures 2–6). Factors V and X are not necessary. The data suggest that platelets also are not necessary. This last finding is different from previous reports (3, 5) which suggest, at least, a partial role of platelets. However, these studies were based on prothrombin consumption (5) and recalcification time (3) techniques. Platelets are necessary for a normal result in both test systems, and thus, it is believed that these systems are not reliable for the study of the development of increased Factor VII activity. The present study does not exclude the possibility that lipoidal materials may be involved. It is possible to conclude only that Hageman factor, PTA, Factors VIII and IX, and calcium are necessary for the development of increased Factor VII activity during the spontaneous coagulation of blood. This finding, which has not been reported previously, suggests that the activation of Factor VII can occur in the absence of tissue thromboplastin. Under these conditions, it depends upon some type of interaction of clotting factors early in the clotting process. It is not known how these factors produce increased Factor VII activity. Studies substituting EDTA for citrate suggest that complex formation may occur which is similar to that seen in studies of Factor VII with tissue thromboplastin (8). Such suggestions, so far, are hypothetical, as many of the factors could not be isolated sufficiently to explore mechanisms and complex formation by utilization of purified factors. However, recent experiments with trypsin (18,

19) show that it is possible to study the development of increased Factor VII activity in some "purified" systems, and this may indicate a new approach.

SUMMARY

Factor VII activity was studied during coagulation of normal and factor deficient blood, in the presence and in the absence of tissue thromboplastin. In normal blood, Factor VII increased its activity two to three times during spontaneous coagulation without tissue thromboplastin. This activity was not due to nonspecific reactions; it was not caused by direct glass activation; and it did not develop in the absence of Factor VII, Hageman factor, PTA, Factors VIII and IX, and calcium. Platelets, Factor V and Factor X did not appear to be necessary for the reaction. Thus, the activation of Factor VII, in the absence of tissue thromboplastin, appears to depend upon an interaction of Hageman Factor, PTA, Factors VIII and IX, and calcium, a finding not reported previously.

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