Studies on the Fate of Coagulation Factors during the Clotting of Normal and Pathological Blood*)

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It is now widely accepted that a final prothrombin conversion factor forms in the absence of tissue juices. This factor is believed to be the end product of a series of intermediate reactions (4, 5, 9, 11, 15, 21) and is usually referred to as "intrinsic blood thromboplastin". The following coagulation factors have been shown to be essential for its formation: Stuart factor (SF, factor XI, Stuart-Prower factor), factor V, platelets, prephase accelerator, AHF (antihemophilic factor, factor VIII), Hageman factor, PTA and factor IX (PTC, Christmas factor).

The scheme shown below (Table 1) which is very similar to Macfarlane's (16) has been suggested by a number of workers (9, 11, 15) for the formation of "intrinsic blood thromboplastin".

(1) Factor IX + AHF + SF + Calcium ⟶ Intermediate Product I.
(2) Intermediate Product I + Platelets ⟶ Intermediate Product II (sedimentable).
(3) Intermediate Product II + Factor V ⟶ Final prothrombin converting substance or "intrinsic blood thromboplastin" (sedimentable).

This scheme is admittedly incomplete as it does not include all the factors involved in the reaction; for example: Hageman factor and PTA are believed to be involved in a phase of clotting preceding the formation of product I (21). Moreover, the scheme does not consider any of the inhibitors known to be important, yet nevertheless it forms a useful working hypothesis. If it is correct, there should be a delay in consumption or utilisation of factor V (involved in Equation 3) during the clotting of blood deficient in any of those coagulation factors involved earlier (Equations 1 and 2). There should

*) This investigation was supported by grant H-3536 from the Nat. Heart Inst., N.I.H. Public Health Service, USA.

also be a failure to utilise AHF in the absence of either factor IX or Stuart factor since the reaction depicted in Equation 1 cannot proceed normally in the absence of any one of these factors. On the other hand, consumption of AHF should be normal in either thrombocytopenia or factor V deficiency. Several such experiments have been performed, but the results appear contradictory. For instance, in factor IX deficiency Douglas (6, 7) found consumption of AHF and factor V to be impaired, while Soulier et al. (21), using a different technique, found consumption of these factors to be normal. Both Soulier et al. (22) and Douglas (6) report normal AHF consumption in thrombocytopenia; on the other hand, Penick (18) states that if platelets are sufficiently reduced so as to delay thrombin production, AHF utilisation is then abnormal. There is evidence indicating that thrombin may be the agent causing disappearance of AHF (1, 18, 19). This suggested a possible explanation of the discrepancies between the work of Douglas (6) and Soulier et al. (22) with respect to utilisation of AHF in factor IX deficiency. The clotting times of the patients with hemophilia B, studied by Douglas, were grossly prolonged with a corresponding lag in thrombin production in contrast to the coagulation times of the factor IX deficient plasmas studied by Soulier which appear to have been only slightly prolonged, if at all. A comparison of AHF utilisation in two hemophilia B subjects who had normal and prolonged clotting times was therefore undertaken; the results are reported here. Factor V consumption studies were also carried out on these subjects. Similar studies were carried out on patients with congenital deficiencies of AHF, SF and factor VII; it might be anticipated, however, that the results in factor VII deficiency would be normal, since this factor is not necessary for the formation of intrinsic thromboplastin.

Johnston, Ferguson, O'Hanlon and Black (14) have studied the fate of factor VII and SF during the clotting of normal blood. They found no change in the level of SF, but factor VII was "activated" so that serum contained 2½ to 3 times the plasma concentration of the factor. The present work includes a study of the fate of factor VII in the absence of SF and vice versa.

Materials and Methods

A patient with congenital factor VII deficiency, two patients with congenital deficiency of Stuart factor, two patients with hemophilia B (congenital factor IX deficiency) and a patient with hemophilia A (congenital deficiency of AHF) were used as subjects in these experiments. One of the patients with congenital factor IX deficiency was a boy aged 9 whose only hemorrhagic symptoms were excessive bleeding following dental extraction and epistaxes;

clotting time and prothrombin consumption were normal; his level of factor IX was assayed and found to be between 10% and 20%. The other patient with hemophilia B was a severe case with a prolonged clotting time and an abnormal prothrombin consumption test. One of the patients with congenital deficiency of Stuart factor has a mild deficiency (approximately 10% SF) with a normal clotting time and was seen by courtesy of Dr. Karl Menk; the other patient, who was seen by courtesy of Dr. Charles Barnett, was originally reported as having factor VII deficiency (2) but is now known to be markedly deficient in Stuart factor (20); the clotting time in this patient is prolonged. The hemophiliac patient was a moderately severe case with less than 2% AHF. The patient with congenital deficiency of factor VII was seen by courtesy of Drs. Harold A. Wurzel of the University of Pennsylvania and C. L. Johnston, Jr. of the University of North Carolina.

All clotting tests were performed in a 37° C water bath in uncoated glass tubes. Uncoated glass syringes were used for the collection of blood, except in the case of the patient with deficiency of factor VII when a silicone-coated syringe was used. The blood from the control for this particular experiment was similarly collected and gave a result well within the normal range; these results are included with those obtained using uncoated syringes. Whole blood was placed in a 10 cm × 1.5 cm uncoated glass tube from which 2.7 ml amounts were rapidly pipetted into a series of 10 cm uncoated glass tubes; these were then incubated at 37° C. One of these tubes contained 0.3 ml 3.8% sodium citrate, and a mixture of the blood and citrate was made immediately after withdrawal of the needle from the vein, and 0.3 ml sodium citrate was added to one of the tubes containing whole blood after exactly 5, 15, 30 and 60 minutes. The contents of each tube were mixed with a wooden applicator stick immediately after the addition of citrate. Each of the five tubes was left in the water bath for a total period of 75 minutes. They were then centrifuged at 3000 r.p.m. (approximately 1500 G.) for 15 minutes and the supernatant citrated plasma or serum separated. The technique described is very similar to that of Douglas (6, 7) but differed in that the tubes in which clotting was arrested were smaller, and a glass pipette was used to transfer the blood samples so that the blood came into more contact with glass surfaces. With this technique clotting, and therefore utilisation of clotting factors, occurs more rapidly than with the original technique of Douglas. The various tests on the pathological bloods were carried out on separate occasions. Altogether 15 different normal controls, including the controls used for the pathological plasmas, were studied.

Stuart factor, factor VII and factor V assays. The technique used for the assay of Stuart factor has been reported elsewhere (10). Similar techniques were employed for the assays of factor V and factor VII, but the substrates were, respectively, stored oxalated plasma and plasma from two patients with congenital deficiency of factor VII. The factor V assays were completed within two hours of the removal of the specimens from the water baths. The AHF assays were performed on the following day. Factor VII assays were performed at various times following collection.

Results

AHF utilisation in congenital deficiencies of factor VII, factor IX and Stuart factor. AHF utilisation was impaired in the patient with the more severe deficiency of factor IX and prolonged clotting time (Figure 1) but was normal in the other patient with the lesser deficiency of PTC and normal clotting time (not shown in Figure 1). Marcacci (17) has reported that utilisation of AHF is abnormal in SF deficiency, and this finding was confirmed in the patient with the more severe congenital deficiency of SF (Figure 1). However, AHF utilisation was normal in the milder case; the latter result is

not shown in Figure 1. Consumption of AHF was normal in the patient with factor VII deficiency (Figure 1). It will be noted that the level of AHF in all experiments dropped precipituously after the first appearance of fibrin (indicated in figure by arrows).

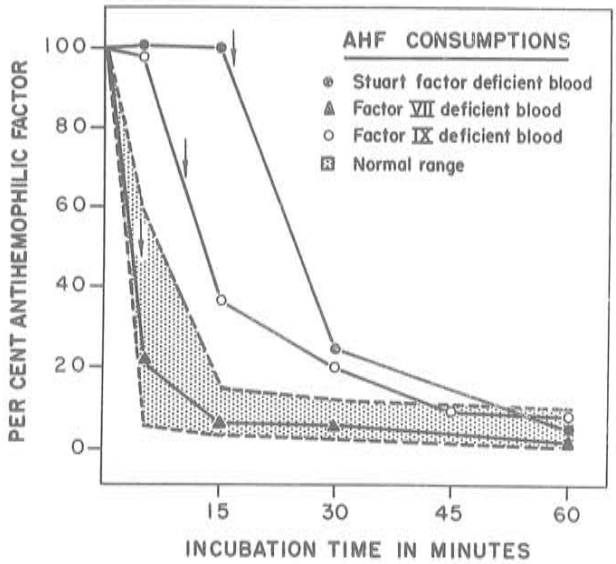

Fig. 1: The concentration of AHF during the clotting of blood from subjects deficient in factor VII, factor IX and SF. The normal range, shown by the dotted area, includes all the results obtained from ten normal subjects. The arrows indicate the time of appearance of the first fibrin strand.

Factor V utilisation in congenital deficiencies of factor VII, AHF, factor IX and SF. The consumption of factor V was found to be impaired in the more severe cases of SF and factor IX deficiencies (Figure 2) but normal in the milder examples of these defects. Consumption of factor V was abnormal in congenital deficiency of AHF, confirming the work of Douglas (7), but normal in factor VII deficiency (Figure 2).

SF levels during clotting of normal and factor VII deficient bloods. Johnston et al. (14) have reported that the level of SF remains constant during the clotting of normal blood. This finding was confirmed. It was also found that the level of SF remained constant during the clotting of the factor VII deficient blood sample.

Two experiments were carried out to determine the level of factor VII during the clotting of blood deficient in SF. In the first experiment no change was found in the level of factor VII during the clotting of either SF deficient

blood or the normal control run in parallel; in the second experiment, a 100% increase in the factor VII concentration of both the SF deficient blood and the normal control was found 30 minutes after collection of the samples. In both of these experiments the specimens were stored at — 20° C for 24 hours before testing.

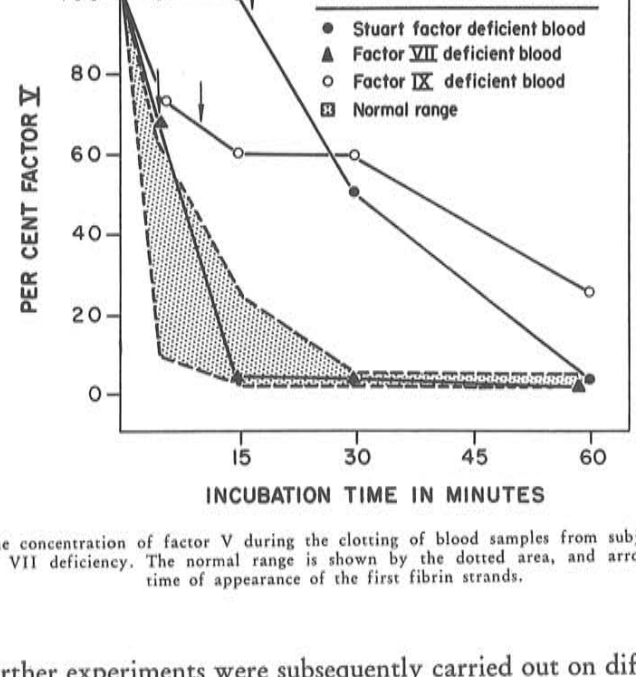

Fig. 2: The concentration of factor V during the clotting of blood samples from subjects with AHF, SF or factor VII deficiency. The normal range is shown by the dotted area, and arrows indicate the time of appearance of the first fibrin strands.

Three further experiments were subsequently carried out on different samples of normal blood (Figure 3). In one of these experiments (Curve C) the assays were commenced immediately; in the other two, the assays were performed one and two hours (Curves A and B, respectively) after removal of the samples from the water bath. It can be seen that in all three experiments the concentration of factor VII increased during clotting. It should be noted that in two of the experiments (Figure 3, Curves B and C) clotting, which is indicated by arrows, occurred just before the first samples (taken 5 minutes after collection of the whole blood) were citrated; the factor VII concentration in these samples was the same as that of the respective plasmas, so that the increase in factor VII concentration occurred after the formation of fibrin. In the third experiment (Curve A) the blood clotted one minute after the first sample was citrated.so that a period of nine minutes elapsed before the next sample was

citrated at the 15 minute incubation period; it is probable, therefore, that in this case also factor VII concentration did not increase until fibrin formed.

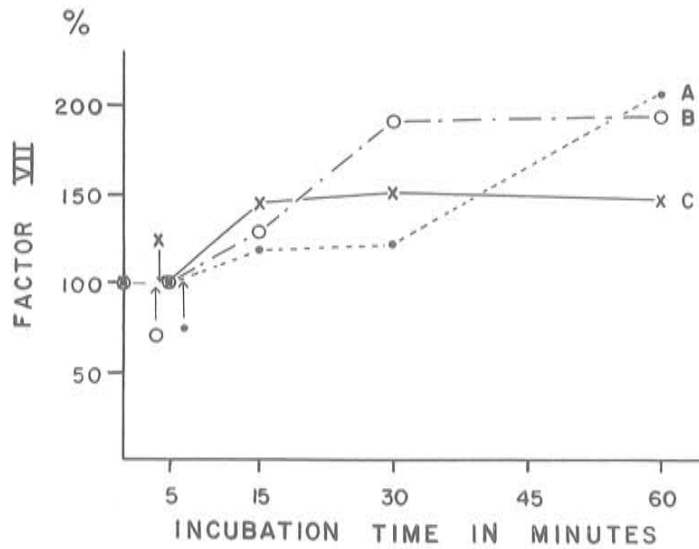

Fig. 3: Concentration of factor VII during the clotting of blood from three normal subjects. The assays were performed immediately (Curve C), one hour (Curve A) and two hours (Curve B) after removing the samples from the water bath. The factor VII level in Curve A was also determined after two hours incubation and was the same as that after one hour incubation.

Discussion

The results reported in the present work suggest that the conflicting results of Soulier (22) and Douglas (6) with respect to AHF utilisation in factor IX deficiency are not due primarily to differences in technique but rather to differences in the coagulation times of the plasmas studied by these workers. When the coagulation time is prolonged, as was the case in Douglas's experiment, utilisation is impaired; on the other hand, if the coagulation time is normal, then AHF utilisation is normal as in Soulier's experiment.

In the experiments reported in this work, utilisation of factor V tended to be slightly slower than that of AHF and was abnormal whenever AHF utilisation was abnormal. The finding that consumption of both AHF and factor V is abnormal only in the more severe cases of SF deficiency and hemophilia B would be expected from the relative crudeness of the techniques and from the observations that consumption of prothrombin is usually normal in patients with very mild hemophilia A or B, or SF deficiency. The findings are in accord with the current concept of blood coagulation (illustrated in the

introduction of this paper) in which a deficiency of factor IX or SF would be expected to result in delayed utilisation of both AHF and factor V, while a deficiency of AHF would result in impaired utilisation of factor V. There are, however, several objections to this interpretation of the present results. Several investigators (1, 18, 19) claim that thrombin is the agent that cause destruction of AHF; if this is true, abnormal consumption of AHF is merely the result of delayed thrombin formation. This view assumes that AHF is an enzyme which is destroyed after it has reacted by a product of coagulation, namely, thrombin. The enzymatic nature of AHF is supported by the work of Bergsagel and Biggs (3) and Surgenor (23) but conflicts with the work of Fisch and Duckert (9).

Penick (18) has found delayed utilisation of AHF in thrombocytopenia and believes that the contrary finding by Douglas (6) of normal utilisation may be explained if the platelet counts in the experiments of Douglas were not reduced sufficiently to cause a significant prolongation of clotting times. In such circumstances I have obtained normal AHF consumptions, but if the clotting time of the platelet-poor plasma was more than 5 minutes longer than the corresponding platelet-rich control plasma, AHF utilisation was impaired (Hougie, unpublished observations). However, Penick's explanation of the discrepancy between his findings and those of Douglas is not entirely satisfactory, for in Douglas's experiment the thrombocytopenia was sufficient to cause delayed utilisation of factor V so that it is probable that the coagulation time was delayed. The finding of Penick of delayed utilisation of AHF in thrombocytopenia is not in accord with the current concept of coagulation, and throws considerable doubt on the validity of this concept. Further observations are needed to resolve this problem. At least it is clear from these remarks that the present findings should not be brought forward as evidence either for or against the modern concept.

The finding that the concentration of SF remains constant during the clotting of normal blood suggests that this factor is an enzyme; this is in agreement with the recent findings of Fisch and Duckert (9). In the present study the concentration of factor VII remained constant until fibrin appeared. This suggests that factor VII, like SF, acts as an enzyme, supporting the more direct experimental evidence of Hougie (1959) and Ferguson, Rierson and Johnston (8). Thus, Hougie (13) has found that factor VII affects rate but not yield of "extrinsic thromboplastin" formation, while Ferguson et al. (8), using a two-stage system, have evidence indicating that factor VII limits rate of thrombin formation without having any effect on final yield, thereby indicating that factor VII is an enzyme. In the present study after clotting occurred, the concentration of factor VII increased; but this

finding was not consistently obtained, and the increase was not as marked as that observed by Johnston, et al. This failure to fully corroborate the findings of Johnston et al., however, might be expected since I used a different type of glassware.*) It is tempting to attribute the apparent increase in the factor VII concentration to the slow destruction of a specific inhibitor of factor VII, but there is no convincing evidence to support this hypothesis. The finding that deficiency of either factor VII or SF in blood has no effect on the fate of the other during coagulation might be expected if both of these factors are, in fact, enzymes rather than substrates.

Summary

In a mild case of Stuart factor (SF) deficiency and in a patient with hemophilia B (factor IX deficiency) consumption of AHF (factor VIII) was normal but was abnormal in more severe examples of these diseases. This finding reconciles previously conflicting reports. Factor V utilisation was abnormal in moderately severe cases of SF deficiency, hemophilia A and hemophilia B but normal in mild cases of SF deficiency and hemophilia B. A mild case of hemophilia A was not studied. These findings would be expected from the modern concept of blood coagulation. However, the findings with respect to AHF are equally well explained if AHF is destroyed by some intermediate product of blood coagulation, such as thrombin, appearing at the time of the appearance of fibrin.

The concentration of SF was found to remain constant during the clotting of both normal blood and blood deficient in factor VII.

The concentration of factor VII during the coagulation of normal blood remained constant until the appearance of fibrin. The concentration then increased, but this finding was not consistently obtained. No abnormality in the fate of factor VII during the clotting of blood deficient in SF was found.

Résumé

Dans un cas bénin de déficience en facteur Stuart et chez un hémophile B (déficience en facteur IX) la consommation du facteur antihémophilique (facteur VIII) est normale. Au contraire dans les cas graves l'utilisation est anormale. Ces résultats expliquent les indications contradictoires rapportées antérieurement.

*) Johnston (personal communication) used new glassware which had not previously been exposed to blood or plasma.

L'utilisation du facteur V est anormale dans les cas de déficience modérée en facteur Stuart, en facteur VIII ou en facteur IX, normale dans les cas de légère déficience. Aucun cas d'hémophilie A bégnine n'a pu être étudié. Les conceptions modernes de la coagulation laissaient prévoir ces résultats. Néanmoins la disparition de l'AHF peut s'expliquer tout aussi bien par l'action de produits intermédiaires, tels que la thrombine, qui apparaissent en même temps que la fibrine.

La concentration du facteur Stuart reste constante pendant la coagulation du sang normal et du sang déficient en facteur VII.

La concentration du facteur VII dans le sang normal reste constante jusqu'à l'apparition de la fibrine. La concentration augmente alors, mais de façon très variable et inconsistante. Aucune anomalie du comportement du facteur VII n'a été constatée durant la coagulation d'un sang déficient en facteur Stuart.

Zusammenfassung

Bei einem Fall von gutartigem Stuart-Faktor-Mangel und bei einem Patienten mit Hämophilie B (Faktor-IX-Mangel) war der Verbrauch des antihämophilen Faktors (Faktor VIII) normal, anormal dagegen bei schwereren Fällen dieser Krankheiten. Diese Resultate erklären frühere, sich widersprechende Mitteilungen. Der Verbrauch des Faktor V war anormal in mäßig schweren Fällen von Stuart-Mangel, Hämophilie A und B, dagegen normal bei gutartigen Fällen von Stuart-Mangel und Hämophilie B. Ein milder Fall von Hämophilie A konnte nicht studiert werden. Die moderne Konzeption der Blutgerinnung läßt diese Resultate erwarten. Trotzdem werden die Resultate bezüglich AHF ebensogut erklärt durch eine Zerstörung des AHF durch irgendein Zwischenprodukt der Gerinnung, wie Thrombin, das zur gleichen Zeit erscheint wie Fibrin.

Es wurde festgestellt, daß die Stuart-Faktor-Konzentration während der Gerinnung sowohl in normalem als auch in Faktor-VII-Mangel-Blut konstant bleibt.

Die Konzentration des Faktors VII blieb während der Gerinnung von normalem Blut konstant bis zum Erscheinen von Fibrin. Dann stieg die Konzentration, allerdings nicht regelmäßig. Es wurde keinerlei Anomalie im Verhalten des Faktors VII während der Gerinnung von Stuart-Mangel-Plasma festgestellt.

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