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The Fate of Factor VII and Stuart Factor During the Clotting of Normal Blood *)

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In 1957, Hougie, Barrow and Graham (1) reported Stuart factor deficiency in a case diagnosed earlier as hypoproconvertinemia (2). Their studies showed that, whereas there were many similarities, the factor lacking was not identical with that of Alexander's patient (3). Subsequent studies, such as those of Bachmann et al. (4) gave additional evidence of the existence of two distinct clotting factors. Thus, many discrepancies in earlier investigations of Factor VII or Factor VII deficiency could be explained.

Factor VII (SPCA or proconvertin) and Stuart factor have a role in prothrombin conversion in the presence of tissue thromboplastin, and are found both in plasma and serum. The present investigation was undertaken to study the fate of each of these factors during the clotting of normal blood. By the use of patients' plasmas as factor deficient substrates, assay specificity for either Factor VII or Stuart factor was assured, since all other clotting factors were present or provided for in normal amounts.

Materials and methods

Whole blood for the clotting studies was drawn from normal, healthy donors (laboratory personnel) into cold siliconized syringes. The blood was distributed quickly into several *new* 13 × 100 mm glass tubes, 1.8 ml per tube. The first tube to which blood was added contained 0.2 ml 0.1 M sodium oxalate and the time of this addition taken as the 0 time. At specific intervals thereafter, 0.2 ml 0.1 M oxalate was added to each of the other tubes, mixed thoroughly, and centrifuged 3000 rpm for 5 minutes. The supernate was kept at 4° C until

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assayed (as quickly as possible) for its content of either Factor VII or Stuart factor by specific assays (see below). In this manner, the concentration of the factors could be studied in specimens before, during, or after clotting occurred. The 0 time specimen served as the control sample containing 100% plasma levels of the factors. Results were obtained for clotting at 4°, 18° and 37° C.

Factor deficient plasmas were obtained from patients with an hemorrhagic diathesis due to deficiency of a specific factor: (a) Stuart deficient (Stuart-) from R. S. (1); Factor VII deficient (VII-) from L. B. (5) made available through the courtesy of Dr. Harold A. Wurzel, Philadelphia. 9 volumes of blood, obtained as above, were anticoagulated with 1 volume 3.2% trisodium citrate and centrifuged 3000 rpm for 10 minutes at 4° C. The resultant plasmas were stored in lusteroid tubes at — 20° C until thawed for use as substrates.

Factor VII and Stuart assays utilized a one-stage clotting technique and differed from each other only in the substrate used. The sample to be assayed was initially diluted 1 : 5 and then mixed with an equal volume of $BaCO_3$ treated bovine serum (AcG) (6), diluted 1 : 50. Both dilutions were made with imidazole-buffered saline (7). Thus, the final test plasma dilution was 1 : 10 and AcG 1 : 100, the latter representing optimal AcG (Factor V) concentration (8). In some experiments, greater plasma dilutions (*i.e.* 1 : 20, 1 : 40) were necessary to obtain clotting-times on the standard curves (see below) for conversion into percentages. 0.1 ml of this diluted mixture was added to 0.1 ml of appropriate factor-free substrate (Stuart — for Stuart, and VII — for Factor VII assay) and clotting at 37° C timed from the addition of 0.1 ml each of thromboplastin (saline-extracted, acetone-dried, human brain powder) and 0.02 M $CaCl_2$. By dilution of normal human plasma, standard curves were constructed for conversion of the obtained clotting-times into percentages (Figures 1 and 2).

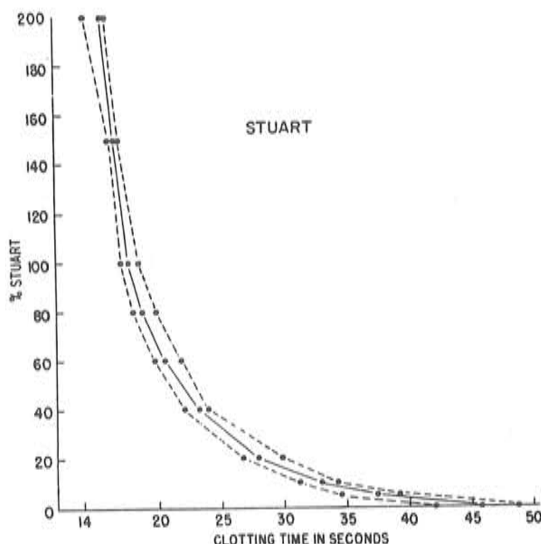

Fig. 1: Reference curve for conversion of clotting-time to percentage Stuart factor; average of 10 normals. Range of clotting-times indicated by broken lines.

Thrombin assay was performed by addition of 0.2 ml undiluted test material to 0.2 ml 1.5% armour bovine fibrinogen (7). The clotting-times obtained were compared with clotting-times obtained upon addition of various dilutions of thrombin (bovine, Upjohn Co.). In this manner, a rough estimate was made of thrombin concentration in the samples tested.

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Results

Reference standards. Figure 1 shows the percentage curves obtained when different concentrations of normal human plasma were tested in the Stuart assay. The clotting-times represent the average results found on dilution of plasmas from 10 normal subjects. The range of the clotting-time values is indicated by the broken lines. Comparable data for the Factor VII assay were obtained and are shown in figure 2. The curves thus constructed served as reference standards for conversion of clotting-times into percentage values.

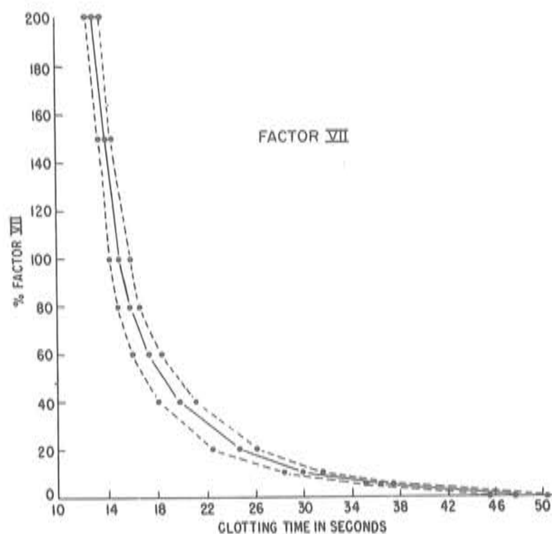

Fig. 2: Reference curve for conversion of clotting-time to percentage Factor VII; average of 10 normals. Range of clotting-times indicated by broken lines.

Fate of Stuart factor. Initially, the blood samples were pipetted into tubes and incubated in a water bath at the temperature indicated until oxalated and centrifuged (see Materials and Methods). Six separate experiments were performed at 37° C, and the average of the results is shown in figure 3a. Additional experiments, one at 18° C (figure 3b) and two at 4° C (figure 3c) are also shown. Minor fluctuations were noted in the early samples in all experiments, and with the possible exception of slight increases in activity (20—30%) in the late 4° C samples, it can be seen that there were no essential changes in Stuart factor concentration. Thus, the level of Stuart factor remained nearly constant during and after clotting of normal blood.

Fate of Factor VII. The same samples obtained for the Stuart assays (see above) were also tested for Factor VII concentration. The results are shown in figure 3d, e, f. In contrast to the Stuart assay data, marked changes were apparent in Factor VII concentration. At the termination of the experiments, the Factor VII concentration was $2^1/_2$ to 3 times that found in plasma. This suggested that "liberation" or "activation" of Factor VII had occurred during clotting. This "activation" was found to vary with the incubation temperature. As might be expected, the slowest rate was found in the 4° C experiments (maximum at 180). Surprisingly, however, the maximum Factor VII concentration was reached most rapidly in the 18° C experiment (40), whereas it was reached considerably later in the 37° C experiment (120). It thus appeared that even though the early stages of "activation" of Factor VII were similar at 18° and 37° C, 18° C was more nearly the optimal temperature for following the fate of Factor VII during clotting.

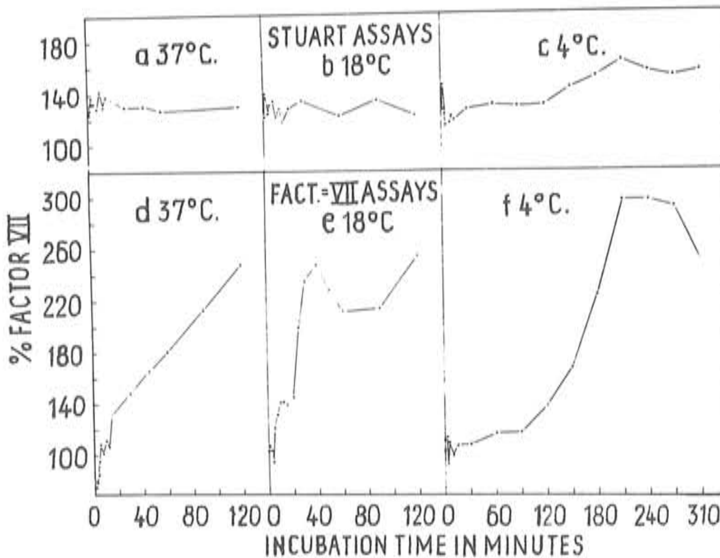

Fig. 3: Concentration of Stuart factor (a, b, c) and Factor VII (d, e, f) before, during, and after the clotting of normal blood, incubated at the temperature indicated.

Tests for thrombin influence of test results. To show that the obtained assay clotting-times were not the result of thrombin in the test mixture, additional experiments were performed. Samples obtained, as for the previous tests, were tested immediately after centrifugation for the presence of thrombin (see Materials and Methods). None of the samples showed any clotting in six hours,

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whereas 0.01 unit of thrombin clotted the standard fibrinogen in 22½ minutes. However, after 24 hours, traces of fibrin had appeared in some of the samples. That such infinitesimal amounts of thrombin did not influence the test results was shown in the following experiment: thrombin was added during the dilution of a plasma sample so that the final test mixture contained 0.1 unit of thrombin (sufficient to clot fibrinogen in 200 seconds). This and a comparable sample without added thrombin, tested in the Factor VII assay, gave clotting-times within 0.3 seconds of each other. Thus, thrombin could not have been a factor in the determinations, since crude tests showed that the samples contained less than 0.01 unit of thrombin.

Discussion

Much of the previous confusion surrounding the reactions of Factor VII and Stuart factor resulted from the use of an artificially prepared substrate supposed to be deficient only in Factor VII. Such was prepared by Seitz filtration of bovine plasma, the precise technique subject to many variations. Both Stuart factor and Factor VII were removed by this technique (1, 4). Attempts to achieve specificity for Stuart factor assay purposes have been made by utilizing thromboplastic lipids with such a substrate (9, 10). However, Seitz filtration may have removed partially other clotting factors as well (11), and activated still others by surface contact (12). Thus, for a study of the individual reactions of Factor VII and Stuart factor, such techniques did not appear to offer the necessary specificity. By substituting specific factor deficient material from human patient sources as substrate, the data obtained were attributable to changes in one factor only.

The present study showed that Stuart factor and Factor VII behaved differently during the clotting of normal blood when specific assays were used to measure their concentrations. Stuart factor levels in serum were found to be comparable to those in plasma. Since no significant changes occurred in Stuart factor concentration during clotting, Owren's suggestion (13) that the presence of convertin and accelerin in fresh serum shortens the clotting-times producing falsely high values was not confirmed. Some of the early fluctuations noted in figure 3a, b, c presumably could have been due to such phenomena, but these did not occur at the time when the concentration of these factors was greatest. Also, incubation temperature changes exerted little, if any, effect on the results. At best, such changes were minor and did not preclude reaching the conclusion that Stuart factor is neither consumed nor activated during clotting. This study does

not add any new information regarding the intrinsic reactions of the Stuart factor in the blood clotting mechanism.

In contrast to Stuart factor, Factor VII serum levels were $2^{1}/_{2}$ to 3 times those found in plasma, thus suggesting that additional Factor VII 'activity' was evolved during clotting. Specific tests showed that these results were not due to thrombin in the test samples. The increase in concentration was regarded as "activation" or "liberation", and figure 3d, e, f shows the rate at which this process took place. Our assay did not distinguish between proconvertin and convertin, and presumably measured the concentration of both. Incubation at 37° C (figure 3d) gave results which, if concentrations of both were added, may be compared to those reported by Owren (13). Thus, validity of data obtained in Factor VII "consumption" studies by use of non-specific assays may be established, but only because it has been shown that the levels of Stuart factor do not change significantly during clotting.

The rate of evolution of Factor VII activity was markedly affected by a change in the sample incubation temperature. As might be expected, incubation at 4° C (figure 3f) slowed the rate, but the final yield was greater than that of experiments performed at other temperatures. Of the temperatures studied, "activation" was most rapid at 18° C although the final yield was comparable to that found on incubation at 37° C. Thus, the optimal temperature for a study of Factor VII reactions may be in an "unphysiologic" range. It is suggested, therefore, that new knowledge may be gained by a careful study of the temperature optimum and reinvestigation of the Factor VII reactions in this light.

Summary

Studies have been presented, utilizing factor specific assays, to show that Factor VII and Stuart factor behave differently during the clotting of normal blood. Stuart factor was neither consumed nor activated during clotting. In contrast, Factor VII was "activated" so that serum contained $2^{1}/_{2}$ to 3 times the plasma concentration of the factor. Marked rate changes occurred in the Factor VII activation curves when the sample incubation temperature was changed. The data obtained suggested that the optimum temperature for Factor VII activation may be at or near 18° C. Previous "consumption" data for "Factor VII" obtained by assays sensitive to changes in both Stuart factor and Factor VII have been validated, since the concentration of Stuart factor does not change during clotting.

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Résumé

Se basant sur les tests spécifiques, il est démontré dans cette étude que les facteurs VII et Stuart se comportent d'une façon différente pendant la coagulation du sang normal. Le facteur Stuart n'est pas consommé ni activé pendant la coagulation. Par contre, l'activité du Facteur VII correspond à 2,5 à 3 fois l'activité du plasma. La rapidité de l'activation du facteur VII est nettement influencée par la température d'incubation. La température optimale pour l'activation du Facteur VII est 18° C.

Les expériences des dernières années sur la consommation du "Facteur VII" dont le taux fut déterminé à l'aide de tests dosant globalement le Facteur VII et le facteur Stuart restent valides, vu que la concentration du facteur Stuart n'est pas modifiée pendant la coagulation.

Zusammenfassung

In der vorliegenden Arbeit wird unter Verwendung spezifischer Teste gezeigt, daß sich Faktor VII und Stuart-Faktor während der Gerinnung von normalem Blut unterschiedlich verhalten. Stuart-Faktor wird während der Gerinnung weder verbraucht noch aktiviert. Im Gegensatz hierzu wird der Faktor VII „aktiviert", so daß das Serum die 2½- bis 3fache Konzentration verglichen mit Plasma enthält. Die Schnelligkeit der Aktivierung von Faktor VII wird durch die Inkubationstemperatur wesentlich beeinflußt. Die optimale Temperatur für die Aktivierung von Faktor VII liegt bei 18° C. Frühere Untersuchungen über den „Verbrauch" von „Faktor VII", die mit Bestimmungsmethoden erhalten worden waren, die zwischen Faktor VII und Stuart-Faktor nicht unterscheiden, behalten ihre Gültigkeit, da sich die Konzentration des Stuart-Faktors während der Gerinnung nicht ändert.

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