Activated Factor VII: Presence in Factor IX Concentrates and Persistence in the Circulation After Infusion

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Factor IX concentrates were evaluated for their factor VII content and for the presence of activated factor VII through use of a coupled amidolytic assay insensitive to activated factor VII and a clotting assay sensitive to activated factor VII. The factor VII content of the concentrates studied (except for one concentrate purposely produced to exclude factor VII) varied between 33 and 621 U/vial. All concentrates contained activated factor VII, as indicated by ratios of factor VII clotting activity to factor VII amidolytic activity, of from 1.6 to 21.5. Higher ratios were found in two brands of activated concentrates than in nonactivated concentrates. In patients infused with factor IX concentrates, plasma factor VII activity rose strikingly in the clotting assay but not in the amidolytic assay. Thus, the elevated factor VII levels by the clotting assay after infusion of factor IX concentrates stem from circulating activated factor VII. A mean intravascular half-disappearance time of 144 min was found for activated factor VII. Its persistence in the circulation makes it important to evaluate the possible role of activated factor VII in the thrombogenicity of factor IX concentrates and in their reported effectiveness in treating bleeding in hemophilia A patients with inhibitors.

The presence of variable amounts of activated clotting factors in factor IX concentrates has hampered accurate measurement of the content of clotting factors in such concentrates, because even small amounts of an activated clotting factor can introduce serious error into test results obtained with conventional clotting factor assays. Factor VII may be present in in vitro materials in at least two forms: as the native one-chain factor VII molecule and, after minimal proteolysis, as a two-chain molecule possessing increased reactivity with tissue factor and therefore referred to as activated factor VII. Some factor IX concentrates, particularly concentrates known to contain large amounts of activated clotting factors (activated factor IX concentrates), have been reported to contain very high levels of factor VII activity, e.g., 6,000 U/vial. Moreover, striking increases in plasma factor VII levels have been reported after infusion of factor IX concentrates into patients. Until now it has not been possible to evaluate the effect on such test results of the presence of activated factor VII in factor IX concentrates.

We have recently described a coupled amidolytic assay for factor VII that is insensitive to the activity state of factor VII. When factor VII is measured both by...
a clotting assay and by this coupled amidolytic assay, it is possible to evaluate the activity state of factor VII in a test material. We report herein the results of such measurements made on different types of factor IX concentrates. In addition, we report the results of measurements of factor VII activity by the clotting and coupled amidolytic techniques in serial samples of plasma from patients infused with factor IX concentrates. It will be shown that factor IX concentrates contain significant amounts of activated factor VII, which is responsible for the marked rise in plasma factor VII activity after infusion of concentrates. Use of these assays has also permitted the determination of the intravascular half-disappearance time of activated factor VII.

**MATERIALS AND METHODS**

Tissue factor was a saline extract of human brain tissue centrifuged two times at 12,000 g to remove large particles. It clotted recalcified normal plasma in 17 sec. Purified factor X was prepared from normal human plasma by a method involving the following steps: barium sulfate or barium citrate absorption, DEAE cellulose chromatography, preparative polyacrylamide gel electrophoresis, and heparin-agarose column chromatography. Tris-buffered saline (TBS) was a solution containing 0.15-M NaCl and 0.05-M Tris, pH 7.5. TBS containing 1 mg/ml bovine serum albumin (TBS-BSA) was prepared with commercially available bovine serum albumin (Sigma, St. Louis, Mo.). A factor VII reference standard, assumed to contain 1 unit (U) of factor VII activity per milliliter, was prepared by pooling plasma from 12 healthy males and was stored in plastic tubes at −20°C. Hereditary factor VII deficiency plasma was obtained from George King Bio-Medical, Salem, N.H. The chromogenic substrate for activated factor X (factor Xa), Bz-Ile-Glx-Gly-Arg-p-nitroanilide (S-2222), was purchased from Ortho Diagnostics, Raritan, N.J.

**Factor IX Concentrates**

Commercial and noncommercial factor IX concentrates were either purchased or provided by the producers during the period January to April 1978. The preparations used were as follows: factor IX complex-human, Proplex (Hyland Laboratories, Costa Mesa, Calif.), lot numbers 581D198AA, 581C206AA, and 581E107AA; Konyne (Cutter Laboratories, Berkeley, Calif.), lot numbers M-6500, M-6418, M-6346, M-3682, and M-5717; Fraction Coagulante P.P.S.B., containing 50 units of heparin, kindly provided by Dr. J. P. Allain from the National Blood Transfusion Center, Paris, France, lot numbers X-125, X-129, X-143, X-215, and X-301; human factor IX concentrate (DE.FIX) kindly provided by Drs. J. Watt and J. Cash from the Scottish National Blood Transfusion Service, Edinburgh, Scotland, lot numbers 251, 314, 324, and 327.

**Activated Factor IX Concentrates**

These were concentrates made for use in treatment of hemophilia A patients with inhibitors that were deliberately prepared to contain increased amounts of activated clotting factors. Auto-IX (Hyland Laboratories, Costa Mesa, Calif.), lot numbers 581D134, 650D007, 650D031, and 650D021, was kindly supplied by Dr. William Thomas in February and March 1978. FEIBA-Immuno (Immuno A.G., Vienna, Austria) was kindly supplied by Dr. F. Elsinger in September 1977. Three lots contained 1,000 FEIBA units per bottle (05A1877, 05A2377, 05A2477); one lot contained 250 FEIBA units per bottle (05A2277).

**Reconstitution and Testing of Concentrates**

Concentrates were reconstituted in the solutions provided by the producers, according to their instructions. At the Hemostasis-Thrombosis Laboratory, San Diego, concentrates were tested for clotting factor activities within about 3 hr after reconstitution. Concentrates were kept in ice until tested. At the Orthopaedic Hospital, Los Angeles, several vials of reconstituted concentrate of the same lot number were pooled for infusion into patients. A subsample was removed immediately after pooling (and before the addition of heparin in those infusions in which heparin was given) and was frozen in dry ice. It was shipped within 3 days in dry ice to the laboratory in San Diego and was stored at −20°C in the laboratory for up to 3 more days before testing.
Protocol for Infusion of Factor IX Concentrates

Informed consent was obtained from each patient. The protocol and the informed consent forms were approved by the human subjects committees of the Orthopedic Hospital, Los Angeles, and the University of California, San Diego. Nine patients with hemophilia B (factor IX levels of less than 0.01 U/ml) and 2 hemophilia A patients with factor VIII inhibitors (2 and 37 Bethesda Units/ml, respectively) participated in the study. Eight hemophilia B patients received their factor IX concentrate (Proplex or Konyne) as one of a series of scheduled infusions of a prophylactic treatment program. One hemophilia B patient received factor IX concentrate (Proplex), and the 2 hemophilia A patients received activated factor IX concentrate (Auto-IX) for the control of bleeding episodes.

In the hemophilia B patients, blood samples were drawn before and at 5, 30, 60, 120, 180, 240, 300, and 360 min and 24 hr after infusion of concentrate. In the inhibitor patients, samples were drawn less frequently. All samples, except the 24 hr sample, were obtained through a 21-gauge needle inserted into the arm opposite that used for the infusion and kept open by a slow drip of isotonic saline. At the time of sampling the saline drip was stopped, 3–5 ml of blood were removed and discarded, and a further sample was removed in a plastic syringe. Nine parts of the sample were added to one part of balanced citrate anticoagulant8 in a plastic tube. Plasma was prepared immediately by centrifugation two times at 12,000 g for 10 min at 4°C and frozen in dry ice. The samples were stored, shipped, and tested as described previously for the concentrates.

Calculation of Observed Rise and Expected Rise in Factor VII Levels After Infusion

The observed rise in factor VII level following infusion was calculated as the difference between the level of factor VII before infusion and the level immediately after infusion. The expected rise in factor VII following infusion was calculated from the formula

\[ \frac{U_{inf}}{W \times 40 \times C.F.} \]

where \( U_{inf} \) is the total units injected as determined from the product of the milliliters injected and the concentration of factor VII (U/ml) in the pooled concentrates, \( W \) is the patient’s weight in kilograms, 40 represents an assumed plasma volume of 40 ml/kg, and C.F. represents a correction factor for the hematocrit. C.F. was calculated as \( (100 - \text{patient’s hematocrit})/(100 - 45) \). This C.F. was used only in 2 patients whose hematocrit values were 33% and 35%, respectively.

Clotting Assay for Factor VII

A one-stage clotting assay for factor VII was carried out by incubating 25 μl of hereditary factor VII deficiency plasma, 50 μl of tissue factor, and 25 μl of adsorbed bovine plasma in a 12- × 75-mm glass tube for 3 min at 37°C. Then 25 μl of a dilution of the test material in TBS and 50 μl of 35-mM CaCl₂ were added, and the clotting time was noted. Since test materials differed markedly in their factor VII activity, different dilutions of test materials were required. Reconstituted concentrates were tested in 1:1000 to 1:5000 dilutions; plasma was tested at 1:10 to 1:100 dilutions. Clotting times were converted to units per milliliter from a dilution curve prepared with 1:10 to 1:80 dilutions in TBS of the factor VII reference standard.

Coupled Amidolytic Assay for Factor VII

An amidolytic assay for factor VII, in which its measurement is coupled to the generation of factor Xa, is described in detail elsewhere.6 It is carried out in two steps. In the first, the test material is incubated with tissue factor, calcium ions, and purified human factor X. The generation of factor Xa is stopped at 3 min by adding Na₂EDTA and cooling. In the second, the factor Xa is measured by adding a subsample of the incubation mixture to an aliquot of the specific chromogenic substrate S-2222 and determining the initial ΔAbs/min in a recording spectrophotometer. Results are converted to factor VII activity (U/ml) from a dilution curve made with 1:80 to 1:400 dilutions in TBS of the factor VII reference standard. Materials were tested in the following dilutions in TBS: for concentrates, 1:500 to 1:2000 dilutions; for plasma, a 1:100 dilution.
Coupled Amidolytic Assay for Factor IXα

An amidolytic assay for factor IXα, coupled to the generation of factor Xα, is described elsewhere. In this assay a test material is incubated with thrombin-activated purified bovine factor VIII, calcium ions, cephalin, and purified human factor X. After 3 min at 37°C the generation of factor Xα is stopped by adding Na2EDTA and cooling. A subsample is assayed for factor Xα activity as described previously. Concentrates were assayed at a dilution in TBS that ranged from about 1:5 to about 1:35; plasma samples were tested undiluted. ΔAbs/min was converted into U/mL of factor IXα from a reference curve prepared with 1:20 to 1:200 dilutions in TBS-BSA of a factor IXα standard. The standard was prepared, each time the assay was carried out, from purified native factor IXα that had been stored at -20°C and that was assumed to have the same factor IX concentration as determined in a clotting assay before storage (5 U/mL). The purified native factor IX was incubated with purified factor XIα and calcium ions for 20 min at 37°C. Under these conditions, 60%-90% of the factor IX was activated to factor IXα as shown by the radioactivity profile after reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis of incubation mixtures containing trace amounts of purified 125I-factor IXα. Therefore, the factor IXα concentration of the standard was calculated by multiplying the final concentration of purified native factor IX in the incubation mixture by 0.75, an assumed mean value for the extent of activation of factor IX to factor IXα.

Amidolytic Assay for Factor Xα

One hundred microliters of test material (undiluted concentrate or undiluted plasma) were added to 600 μL of TBS and 100 μL of S-2222 (1 mg/mL), and the initial ΔAbs/min was measured. Details of the assay are given elsewhere. A factor Xα reference standard was prepared each day that the test was carried out by incubation for 5 min at 37°C in plastic tubes of 200 μL of a purified factor X preparation (final concentration 1.1 U/mL), 20 μL of a 66-μg/mL solution in TBS of Russell’s viper venom, and 20 μL of 50-mM CaCl2. Then 20 μL of 0.3-M Na2EDTA were added, and the tubes were transferred to ice. A reference curve was prepared with 1:7.5 to 1:40 dilutions of this incubation mixture. Later we discovered that a 5-min incubation period under our conditions resulted in only 33% conversion of the factor X preparation to factor Xα. Consequently, the factor Xα concentrations as read from the dilution curves, which presumed full activation of the reference standard, were divided by 3.

RESULTS

Factor VII Activity in Concentrates

The data on the factor VII activity of factor IX concentrates and activated factor IX concentrates are summarized in Table 1. All brands of concentrate studied except DE.FIX contained appreciable amounts of factor VII (33–621 U/vial), as measured by the coupled amidolytic assay. DE.FIX contained only from 2.9 to 3.6 U of factor VII per vial. In every concentrate studied, a higher value for factor VII was found in the clotting assay than in the coupled amidolytic assay. The ratio of

| Table 1. Factor VII Activity in Factor IX Concentrates and Activated Factor IX Concentrates |
|-----------------------------------|-----------------|-----------------|-----------------|
| **Factor IX Concentrates**        | **Range of Factor VII Activity per Vial** | **Ratio VII/VIIα** |
| Brand                             | No. Lots Tested | Clotting Units (VIIu) | Amidolytic Units (VIIαu) |                  |
| Konyne (USA)                      | 5              | 294–618           | 54–139            | 4.4–9.1          |
| Proplex (USA)                     | 3              | 1338–3006        | 158–621           | 4.4–8.5          |
| P.P.S.B. (France)                 | 5              | 123–390         | 40–136            | 2.9–3.8          |
| DE.FIX (Scotland)                 | 4              | 4.8–6.6         | 2.9–3.6           | 1.6–2.1          |
| Activated Factor IX Concentrates  |                 |                  |                  |                  |
| Auto-IX (USA)                     | 4              | 1725–3330        | 117–266           | 6.8–21.5         |
| FEIBA 1000 (Austria)              | 3              | 960–2310         | 70–165            | 13.7–14.4        |
| FEIBA 250 (Austria)               | 1              | 480             | 33                | 14.4             |

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factor VII activity in the clotting assay to factor VII activity in the coupled amidolytic assay (VIIc/VIIam) varied from 1.6 to 21.5. Significantly higher values (p < 0.01) for this ratio were obtained from activated factor IX concentrates (mean 13.9 ± 1.6 SE) than from nonactivated factor IX concentrates (mean 4.24 ± 0.6 SE).

**Factor IXa and Factor Xa Activities in Concentrates**

Only small amounts of factor IXa activity were found in nonactivated factor IX concentrates: from 0.4 to 3.4 U/vial in three lots of Konyn, from 0 to 1.1 U/vial in three lots of Proplex, from 0.1 to 0.5 U/vial in five lots of P.P.S.B., and 0.6 U/vial in four lots of DE.FIX. The activated factor IX concentrates appeared to contain considerably higher factor IXa activity, but quantitative data were not reliable because different dilutions of an activated concentrate were found to yield significantly different quantitative values in our assay.

No factor Xa activity could be measured in six lots of nonactivated factor IX concentrates. Two lots of Konyn contained traces of factor Xa activity, about 0.1 U/vial. One lot of an activated factor IX concentrate had no measurable factor Xa activity. Very small amounts of factor Xa activity, from about 0.1 to 0.6 U/vial, were found in seven other lots of activated factor IX concentrates.

**Factor VII Activity in Plasma Following Infusion of Factor IX Concentrates**

Ten infusion studies were carried out in 9 hemophilia B patients. Mean values ± SE for factor VII activity in these patients before infusion were as follows: in the clotting assay, 0.73 ± 0.06 U/ml; in the coupled amidolytic assay, 1.28 ± 0.09 U/ml. Immediately after infusion, the mean values were as follows: in the clotting assay, 3.49 ± 0.77 U/ml; in the coupled amidolytic assay, 1.44 ± 0.01 U/ml. The elevated factor VII activity found in the clotting assay gradually fell over a period of hours to the activity found before infusion (Fig. 1).

In all of the infusion studies, the observed rise in factor VII activity, as measured in the clotting assay, exceeded the expected rise in factor VII activity (Table 2). A larger difference between mean observed rise and mean expected rise was found with infusion of Proplex than with infusion of Konyn. A comparison between observed and expected rises in the coupled amidolytic assay was meaningless because of the small changes in factor VII activity found by this assay technique.

![Fig. 1. Plasma factor VII activity levels, as measured in the clotting assay (VIIc) and coupled amidolytic assay (VIIam), following infusion of factor IX concentrates. Each point is the mean for 10 infusions.](image-url)
Table 2. Expected vs. Observed Rise in Factor VII Activity in the Clotting Assay Following Infusion of Factor IX Concentrates and Activated Factor IX Concentrates

<table>
<thead>
<tr>
<th>Patients*</th>
<th>Total Dose (U)</th>
<th>Expected Rise (U/ml)</th>
<th>Observed Rise (U/ml)</th>
<th>Observed/Expected Rise</th>
<th>Difference Between Observed and Expected Rise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia B†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Konyne (6)</td>
<td>988-3024</td>
<td>0.68 ± 0.34</td>
<td>1.01 ± 0.42</td>
<td>1.55 ± 0.23</td>
<td>1.498</td>
</tr>
<tr>
<td>Proplex (5)</td>
<td>5104-9600</td>
<td>1.90 ± 0.73</td>
<td>4.71 ± 2.25</td>
<td>2.49 ± 0.51</td>
<td>2.658</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>with inhibitors to factor VIII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auto-IX (2)</td>
<td>5229, 5278</td>
<td>5.09, 5.23</td>
<td>6.86, 6.36</td>
<td>1.37, 1.22</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses denote numbers of patients studied.
†Data from a total of 11 infusions with factor IX concentrates are given because of inclusion of data from an additional infusion in which factor VII activity was followed for 1 hr only.

Two patients with hemophilia A and an inhibitor to factor VIII were given an activated factor IX concentrate (Auto-IX) on two consecutive days during a bleeding episode. Very high factor VII levels in the clotting assay were observed in these patients after each infusion: in one patient up to 8.6 U/ml and 7.6 U/ml and in the other patient up to 8.0 U/ml and 7.6 U/ml. As in the hemophilia B patients, only a slight rise in factor VII activity was measured in the coupled amidolytic assay.

The individual values for factor VII clotting activity used to calculate the data points of the descending slope of factor VII activity shown in Fig. 1 were converted into percentages of the mean observed rise in factor VII clotting activity. The mean values for these percentages are plotted on semilogarithmic paper against time in Fig. 2. The points could be fitted to the biexponential equation

\[ C_t = A_1e^{\alpha_1 t} + A_2e^{\alpha_2 t} \]

in which \( A_1 \) and \( A_2 \) are the intercepts (exponential coefficients) and \( \alpha_1 \) and \( \alpha_2 \) are the slopes (exponential rate constants). Their computed values were as follows: \( A_1 = 18.6, A_2 = 81.4, \alpha_1 = 2.1, \alpha_2 = 0.29 \). The mean intravascular half-disappearance time of the infused factor VII clotting activity, calculated from \( \alpha_2 \), was 2.4 hr (144 min).

In 6 of the 10 infusion studies in hemophilia B patients, heparin (300 IU) was added to the pooled concentrate immediately before infusion. No difference in initial rise in factor VII clotting activity or in its rate of disappearance was noted between infusions given with heparin and infusions given without heparin.

**Factor IX<sub>a</sub> and Factor X<sub>a</sub> Activities in Plasma Following Infusion of Factor IX Concentrates**

As expected from the small amounts of factor IX<sub>a</sub> present in the factor IX concentrates, either no factor IX<sub>a</sub> or only traces of factor IX<sub>a</sub> could be measured in the plasma from 8 patients with hemophilia B immediately after infusion. Moreover, only traces of factor IX<sub>a</sub> were detectable immediately after infusion in the plasma of the 2 inhibitor patients given activated factor IX concentrate without heparin. Despite the use of undiluted plasma as the test material in these studies,
Fig. 2. A plot of the rate of disappearance of factor VII clotting activity after infusion of factor IX concentrates. Each point represents the mean of values from 10 infusions, expressed as percentage of the mean initial observed rise (the difference between the clotting factor VII activities before infusion and after infusion).

The activity measured approached the limits of sensitivity of the assay. No plasma factor Xa activity was measurable after infusion of concentrates.

DISCUSSION

A factor IX concentrate containing activated factor VII will have a higher apparent factor VII content as measured in a one-stage factor VII clotting assay than as measured in the coupled amidolytic assay for factor VII. This discrepancy is found because activated factor VII gives shorter clotting times in the factor VII clotting assay than does native factor VII, whereas test results in the coupled amidolytic assay are not affected by the activity state of factor VII. The ratio of the activity measured in the clotting assay to the activity measured in the coupled amidolytic assay (factor VII activity ratio) will reflect the extent of activation of factor VII in the concentrate.

The data obtained with the coupled amidolytic assay establish that all concentrates studied except DE.FIX (from which factor VII had been deliberately removed) contained appreciable amounts of factor VII, i.e., from 33 to 621 U/vial. Amounts varied between different brands of concentrates but also between different lots of the same brand of concentrate (Table I). Activated factor IX concentrates appeared to contain similar amounts of factor VII as did nonactivated factor IX concentrates. Every concentrate studied contained activated factor VII,
as evidenced by factor VII activity ratios that varied between 1.6 and 21.5. The factor VII activity ratios of activated factor IX concentrates significantly exceeded the factor VII activity ratios of nonactivated concentrates (Table 1).

Although the factor VII activity ratio allows comparison of the relative amounts of activated factor VII in different concentrates, it does not provide a measure of the concentration of activated factor VII molecules in a concentrate. The latter data will not be obtainable until purified human factor VII can be activated completely with a purified human factor VII activator and the molecular changes so induced can be correlated with the increase in reactivity found in a factor VII clotting assay.

It is noteworthy, however, that Radcliffe and associates have reported a 35-fold increase in factor VII clotting activity induced by the repeated addition of a preparation of activated factor XII to human plasma. Thus, even a factor VII activity ratio of 21.5, the highest ratio found in our study, probably reflects the activation of only a portion of the factor VII molecules in that concentrate.

The infusion studies, which revealed a striking difference after infusion between plasma factor VII activity as measured in the clotting assay and in the coupled amidolytic assay (Fig. 1), established that the marked rise in plasma factor VII level measurable in a clotting assay after infusion of factor IX concentrate results from circulating activated factor VII. Activated factor VII persisted in the circulation after infusion with a mean intravascular half-disappearance time of 144 min (Fig. 2). The data in Fig. 2 in the paper of Kurczynski and Penner may now also be interpreted as evidence of the persistence over several hours of activated factor VII in the circulation of their patient infused with an activated prothrombin complex concentrate. In addition, the data of Hoag et al., who reported intravascular half-disappearance times of 85–120 min in four severe hereditary factor-VII-deficient patients infused with an early experimental concentrate, probably represent evidence for the disappearance over a period of hours of activated factor VII from the circulation. These observations fit with the recent demonstration that antithrombin III, in contrast to its neutralization of other activated serine protease clotting factors, is unable to neutralize activated factor VII. The data also mean that activated factor VII, as opposed to factor IX, is not cleared immediately from the circulation by a cellular clearance mechanism in the liver.

Half-disappearance times of 290–355 min have been reported for plasma factor VII activity in normal subjects given huge doses of oral anticoagulants to block normal factor VII synthesis. If these values represent the intravascular half-disappearance times of native factor VII, then our finding of a mean intravascular half-disappearance time of 144 min for activated factor VII suggests that the activated factor disappears from the circulation at about twice the rate of the native factor.

We cannot explain why the observed rises in plasma factor VII clotting activity after infusion exceeded by up to threefold the expected rises in factor VII clotting activity as calculated from the clotting activity infused and an assumed plasma volume of 40 ml/kg. Hoag et al. reported similar findings following the injection of an experimental concentrate into a normal subject and into patients with moderate factor VII deficiency. The discrepancy could conceivably reflect activation of endogenous factor VII by an activity in the concentrate. We suspect, however, that it reflects underestimation of the factor VII clotting activity of the concentrates.
related to their high dilution prior to assay in buffer. (In a supplemental experiment, a concentrate was diluted 1:1000 in TBS, in TBS-BSA, and in a 1:100 dilution of hereditary factor-VII-deficient plasma. The following values were found for factor VII activity in the clotting assay: for the dilution in TBS, 95 U/ml; for the dilution in TBS-BSA, 125 U/ml; for the dilution in factor-VII-deficient plasma, 160 U/ml.)

The low values for factor IXa activity that we found in nonactivated concentrates differ remarkably from the very much higher values for factor IXa activity in nonactivated concentrates reported by Elődi and Varadi, who used a clotting assay for factor IXa. The absence of factor IXa or the negligible amounts of factor Xa that we found in nonactivated concentrates and the very small amount of factor Xa that we found in activated concentrates are also much less than the values for this activated factor reported by Elődi and Varadi. Our data on factor IXa and factor Xa activity in concentrates should be considered approximations in view of the absence of uniform reproducible reference standards for either factor IXa or factor Xa activity.

Neither factor IXa nor factor Xa could be detected by our assay techniques in the plasma of patients infused (with or without added heparin) with either nonactivated or activated concentrates. This finding contrasted strikingly with the ready demonstration of circulating activated factor VII after infusion.

The persistence of activated factor VII in the blood for some hours after infusion of factor IX concentrates highlights the need to evaluate the potential thrombogenicity of activated factor VII in concentrates. Clearly, in the absence of exposure to tissue factor, activated factor VII should not increase blood coagulability. However, in the presence of a low concentration of tissue factor, activated factor VII will shorten the clotting time of blood (e.g., a 1:50 dilution of our tissue factor preparation clotted plasma containing native factor VII in 85 sec and clotted plasma containing activated factor VII in 60 sec). Consequently, in clinical situations where blood might be exposed to tissue factor, e.g., in a patient with hemophilia B during surgery or following trauma, the infusion of activated factor VII in concentrates could, at least theoretically, increase the risk of initiating a thrombotic episode.

Test methods presently used to evaluate the thrombogenicity of factor IX concentrates are not sensitive to variation in the factor VII activity of concentrates. The nonactivated partial thromboplastin time, which has been advocated as an in vitro test for detecting thrombogenic material in concentrates, is an intrinsic clotting test independent of factor VII activity. Moreover, the model for stasis thrombosis developed by Wessler et al., which has been used as an in vivo test for the thrombogenicity of concentrates, probably does not reflect the effect of activated factor VII, since this model is based on clotting static venous blood in the presumed absence of vascular trauma and resultant exposure of the blood to tissue factor. Consequently, we believe that measurement of the factor VII activity ratio of concentrates deserves evaluation as an additional indicator of their potential thrombogenicity.

The activity (or activities) responsible for the reported effectiveness of activated factor IX concentrates in controlling bleeding in a hemophilia A patient with inhibitors to factor VII has not been identified. Whether or not the increased amount of activated factor VII in such concentrates contributes to their hemostatic
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Effectiveness is an open question. Patients with hemophilia bleed because clotting initiated by tissue factor cannot compensate for their defect in intrinsic clotting. Nevertheless, this reasoning does not exclude, a priori, the possibility that substantial amounts of activated factor VII in the circulating blood could exert a hemostatic effect in a hemophilic patient with an inhibitor in the presence of tissue factor at a bleeding site. It would be of interest, in clinical trials of activated factor IX concentrates in the management of bleeding in such patients, to attempt to relate serial factor VII activity ratios to clinical response.

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