amplified by interaction with the calcium ion in the bloodstream of the patient being treated.

Other experiments have shown that antithrombin III plus heparin can inhibit either the thrombogenic materials found in concentrates, or the enzymes generated de novo by prior incubation with calcium.

G. H. Tischkoff (American Red Cross, Lansing, Michigan, United States): Prothrombin-Complex Concentrates, Thromboses, and Factor VIII Inhibitors: Evidence for a Coagulant Formed by the Interaction of Factors X and II.

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Kureczynski and Penner have reported on the use of prothrombin concentrates in the management of bleeding in hemophilia patients with Factor VIII inhibitor. Several commercial concentrates showed marked ability to shorten the activated partial thromboplastin time (APTT) of Factor VIII inhibitor plasma after 40 min preincubation. Significantly, purified human Factor Xa and thrombin did not evidence corrective effect. Highly purified human Factors X and II, isolated by affinity chromatography from partially activated prothrombin complex, effectively corrected the APTT when combined, but were inactive when tested separately. Preliminary studies suggest that Factor V is essential. This study indicates that the thrombogenic properties of prothrombin concentrates in vivo may be due in part to a coagulant complex formed by the interaction of X, V, and a prothrombin intermediate.


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Well documented thrombotic side effects of II, VII, IX and X concentrates are thought to be due to spontaneous partial activation of one or more coagulation factors. Using the unactivated PTT method of Kingdon and the thrombin time as global assays of activation, we have confirmed this concept. It has been suggested that heparin should be added to the concentrate, but preparations vary in antithrombin content and activated factor. The amount of heparin for each preparation would have to be titrated in order to neutralize the activated factors without anticoagulating the patient. We sought to extend the range of added heparin without producing an anticoagulant effect, by adding heparin to the concentrates prior to use of the PEG fractionation method to remove the hepatitis antigen. Thus, heparin-activated antithrombin III can inhibit activated clotting factors while residual free heparin is removed by PEG fractionation. When 1 unit of heparin per mg of protein in the concentrate was added prior to PEG treatment, the final products from more than 80 runs on 15 batches (3 major manufacturers) were almost invariably neutral; neither anticoagulated nor activated. The thrombin clotting time, when measured, was 6-24 hours. The average yield with use of the PEG fractionation method was 83%; depending on the initial purity of the concentrate, the final product had an additional purification of 1.0-2.5× that of the starting material.

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The effect of some variables on the yield of factor VIII procoagulant activity during the preparation of factor VIII concentrates was investigated. The results may be summarized as follows:

- The pH of the anticoagulant solution, which results in an optimal recovery of factor VIII in plasma varies with the time, during which the blood or plasma has to be stored before the plasma is frozen. The temperature during the storage of blood and plasma should be higher than 10°C.
A high freezing and thawing rate of the plasma results in a high recovery of factor VIII and less contaminating proteins and lipids in the cryoprecipitate.

After centrifugation and dissolving of the precipitate, the preparation may be lyophilized. An appreciable loss of activity is found when the temperature during this process exceeds 20°C.

From the cryoprecipitate factor VIII can be further purified by precipitation with PEG 4000. The fraction between 2%/4% at 20°C and 6% at 0°C was used. Within certain limits this precipitation is independent on the protein concentration, pH, time, and ionic strength. Mandelate (0.3% w/v) prevents irreversible precipitation of fibrinogen during all operations.

Sterilization of the dissolved precipitate was achieved by filtration through membrane filters (0.3 μm) on which the filter aids Celite 535 and Hyflo supercel were layered.

PEG precipitation and sterilization induce a 20% loss.

The clinical use of the product was assayed in series of infusions in hemophilics.


Standard Cryoprecipitate was prepared from fresh citrate phosphatedextrose plasma by snap freezing at −70°C and then thawing at +4°C in air for 18 hours. In 143 experiments the yield of Factor VIII from the starting plasma was 42%.

In 64 paired experiments the Factor VIII yield in Cryoprecipitate from fresh plasma was increased, from 43%, in the standard method to 56%, when a quick thaw of 50 minutes at +4°C in a liquid bath was introduced. In 10 other paired experiments the yield in the standard method was raised from 51% to 61% when 90 minutes of super-cooling at −6°C in a liquid bath was introduced prior to snap freezing. When, however, the quick thaw and super-cooling modifications were combined in 42 paired experiments, the yield was only 49%, compared with 42% by the standard method.

It is concluded that this simple quick thaw modification will produce a greater yield of Factor VIII in Cryoprecipitate and that the addition of the technically more demanding super-cooling modification does not give a significantly greater yield.

It seems likely that the longer period at +4°C in the standard method leads to denaturation of a proportion of the Factor VIII and loss of activity. Factor VIII antigen, however, was not lost. In a smaller number of experiments approximately all the Factor VIII was recovered in the Cryoprecipitate and its supernatant. Furthermore, the relative proportions of Factor VIII antigen and procoagulant in the Cryoprecipitate were found to vary in concert suggesting that the Factor VIII molecule is not dissociated in the process of cryoprecipitation.


SDS gel electrophoretic analysis of the products of human prothrombin (ΠH) activation reveal a similar product distribution to that observed for bovine prothrombin (ΠB). However, some subtle differences are noted when the amino-terminal sequences of the activation intermediates are determined. The amino-terminal sequences of ΠH and ΠH intermediate 3 are identical (ALA, ASN, PRO, PHE, LEU, GLU, GLU, VAL, ARG, LYS) and homologous to the sequence for ΠB. Similarly the amino-terminal sequences of ΠH intermediate 1 and ΠH intermediate 4 (SER, GLU, GLY, SER, SER, VAL, ASN, LEU, SER, PRO) are entirely homologous with their bovine counterparts. On the other hand, ΠH intermediate 2 and α-thrombin A chain have the sequence THR, PHE, GLY, SER, GLY, GLU, ALA, ASN and this sequence is homologous to the ΠB intermediate 2, α ΠB A chain beginning with residue 14. Studies of the cleavage of ΠH-intermediate 1 with factor Xa in the presence of DFP and Hirudin and with thrombin in the presence of soybean trypsin inhibitor indicate that the ΠH intermediate 2 originally produced by