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Note

Isolation of factor IX concentrates for clinical use by ion-exchange chromatography and ammonium sulphate precipitation

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Prothrombin complex factors are isolated usually by adsorbing on ion-exchangers[1,2]. The subsequent elution step provides solutions of moderate concentration, which, in addition, often have to be desalted[2,3]. However, further concentration by intermediate lyophilization[2–4] or alcohol precipitation[5] is expensive and time-consuming or detrimental[6]. We have therefore introduced ammonium sulphate as a concentrating agent.

EXPERIMENTAL

(A) Details of our standard procedure with DEAE-Sephadex as adsorbent are given in Scheme 1.

(B) In an alternative method, we used DEAE-cellulose 130 gr (Schleicher and Schüll). In this case, the prothrombin complex was eluted with buffers of lower ionic strength in columns of 100 × 21.5 cm I.D. (Pharmacia) or 50 × 18 cm I.D. (Amicon), respectively. Moreover, the first precipitation with ammonium sulphate was ommitted.

In both methods, heparin was added at the beginning and end of the process.

Assays (selected)

Activities of clotting factors II, VII, IX and X were determined by the one-stage method using the reagents of Merz and Dade. Tests for thrombogenicity and activated factors (Xa, thrombin) were performed as described by Kingdon[7] and with chromogenic substrate S 2222 (Kabi) or Chromozym TH (Boehringer, Mannheim, F.R.G.), respectively.

RESULTS

Purification of factor IX is *ca.* 220-fold and the yield is 45–55% with respect to the supernatant of cryoprecipitate. Final products are readily soluble, free of thrombogenicity or activated factors and contain 50–60 U of factors II, IX, X and *ca.* 16 mg/ml protein. Factor VII is poorly adsorbed under our experimental conditions. Most of its activity is found in the wash and first precipitate with ammonium sulphate. Procedure B yields less contaminating proteins than procedure A, as revealed by polyacrylamide gel electrophoresis. The concentrates can be heated in the

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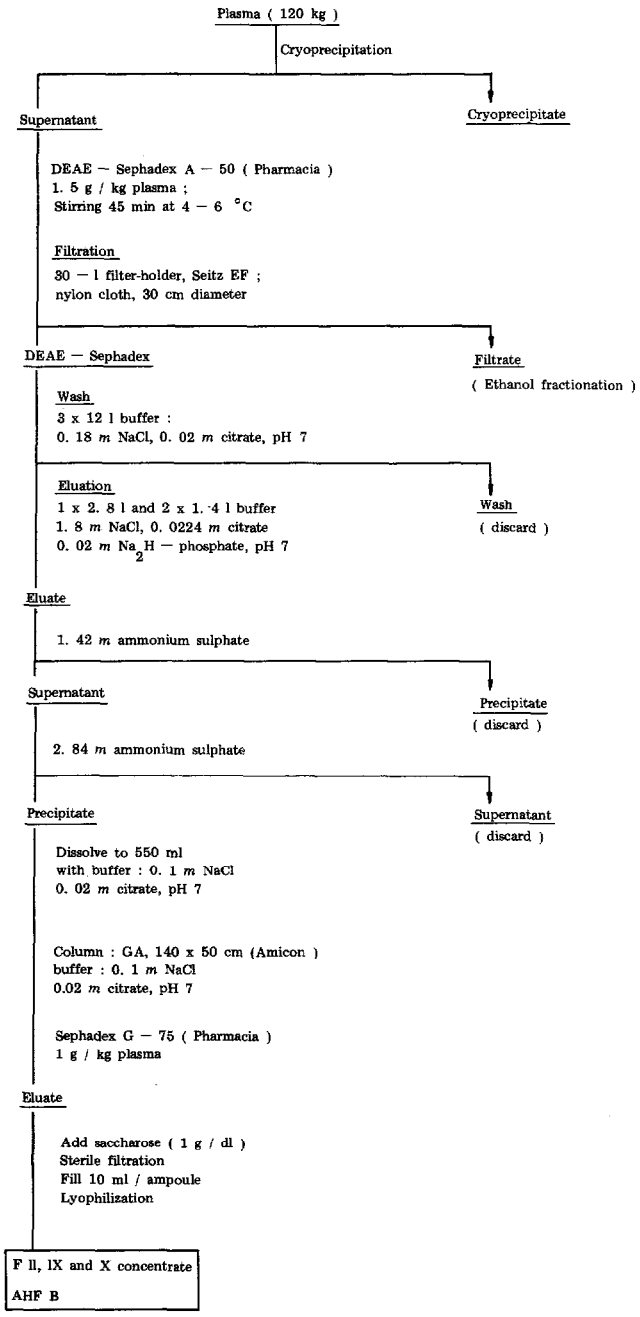
Plasma (120 kg)
Cryoprecipitation
Supernatant
Cryoprecipitate
DEAE — Sephadex A — 50 (Pharmacia)
1. 5 g / kg plasma ;
Stirring 45 min at 4 — 6 °C
Filtration
30 — l filter-holder, Seitz EF ;
nylon cloth, 30 cm diameter
DEAE — Sephadex
Filtrate
(Ethanol fractionation)
Wash
3 x 12 l buffer :
0. 18 m NaCl, 0. 02 m citrate, pH 7
Elution
1 x 2. 8 l and 2 x 1. 4 l buffer
1. 8 m NaCl, 0. 0224 m citrate
0. 02 m Na2H — phosphate, pH 7
Wash
(discard)
Eluate
1. 42 m ammonium sulphate
Supernatant
Precipitate
(discard)
2. 84 m ammonium sulphate
Precipitate
Supernatant
(discard)
Dissolve to 550 ml
with buffer : 0. 1 m NaCl
0. 02 m citrate, pH 7
Column : GA, 140 x 50 cm (Amicon)
buffer : 0. 1 m NaCl
0.02 m citrate, pH 7
Sephadex G — 75 (Pharmacia)
1 g / kg plasma
Eluate
Add saccharose (1 g / dl)
Sterile filtration
Fill 10 ml / ampoule
Lyophilization
F II, IX and X concentrate
AHF B

Scheme 1.

lyophilized state for up to 96 h at 70°C without perceptible changes in composition and activity.

DISCUSSION

Procedure A in particular is versatile and can be scaled up to larger volumes of plasma. Vessels of special design for the concentrating elution of DEAE-Sephadex[8] are not required. In the eluate, the ionic strength can be varied over a vast range. Thus, small elution volumes are possible. Precipitation by ammonium sulphate is, to some extent, insensitive to temperature and stabilizes proteins. Hence, precipitates can be stored and then combined for desalting in larger batches. Sephadex G-75 as a separation medium proved to be better than Sephadex G-25[9]. Respective gel bed heights of 18 and 21 cm allow short running times. The process is compatible with ethanol fractionation and other methods for the isolation of plasma proteins.

With method B and DEAE-cellulose as adsorbent we found pyrogen-like reactions in rabbits from time to time. This and the material's tendency to clog to the nylon gauze led us to use DEAE-Sephadex in routine preparations for clinical use,.

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