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BRIEF COMMUNICATION

THROMBOGENICITY OF FACTOR IX CONCENTRATES AND POLYETHYLENE GLYCOL PROCESSING

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INTRODUCTION

The major problems concerning the clinical use of factor IX concentrates are their potential for transmitting hepatitis (1, 2) and the possibility of thrombogenic complications in the recipient (3, 4).

At the Protein Fractionation Centre of the Scottish National Blood Transfusion Service a concentrate of coagulation factors II, IX and X (DEF IX) is prepared using DEAE-cellulose (5). This preparation has been used as a model starting material in a study of the removal of hepatitis B surface antigen [illegible] factor concentrates by [illegible] PEG 4.

[illegible] batches of DEF IX have been [illegible] this means. [illegible] these batches had failed to meet our in-house [illegible] control standard for thrombogenicity however, results from current in vitro tests suggest that this potentially thrombogenic material may have been removed by PEG processing. These observations are presented here. Other details of the process and product development will be presented elsewhere.

MATERIALS AND METHODS

Materials: Factor II, IX and X concentrate for processing was obtained by pooling eluates of the factors prepared by DEAE-cellulose adsorption from citrated, factor VIII-depleted plasma (5). PEG 4000 was supplied by Union Carbide (U.K.) Limited.

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Analytical Methods: Factor II was measured by the method of Jobin and Esnouf (8). Factor X was estimated according to Denson (9) and factor IX by the one-stage method of Denson and Biggs (10). Thrombin content was estimated by the thrombin-fibrinogen clotting time as previously described (5), but calibrated against thrombin solutions containing appropriate concentrations of PEG and protein (11). PEG concentration was measured according to the method of Childs (12).

Potentially thrombogenic material was assayed in vitro by the non-activated partial thromboplastin time (NAPTT) (3), also expressed as the ratio of sample to control (NAPTR), and the thrombin generation time (TGt50) according to Sas et al. (13) and modified by the addition of factor V (0.25 u/ml) and phospholipid (0.1 ml platelet substitute) (14). Factor Xa generation was measured spectrophotometrically using the synthetic polypeptide S2222 (Kabi Vitrum) in the semi-micro assay of Pepper et al. (15) with addition of phospholipid (0.1 ml platelet substitute) (14). The results are expressed as the incubation time required for an absorbance increase of 0.5 (Δ OD = 0.5). Defibrinated NAPTT control plasma (NAPTT 253 seconds) was used to provide a reference point as the other materials tested are manufactured concentrates which could vary from lot to lot.

Preparative Methods: The fractional precipitation of DEFIX eluates was carried out as shown in fig. 1. This is similar to the scheme previously described (7), but heparin was not added, either during or after processing. Precipitation of factors II, IX and X into P2 has been optimised at a sodium chloride concentration of 0.2 moles/l without the use of albumin as a co-precipitant. Precipitate P2, in 30% PEG, was resuspended in 20% PEG to reduce the concentration of PEG in the final product. DEFIX batches processed in this way were then pooled to form Supernine batches of a suitable volume, the final product being sterilised by membrane filtration, dispensed in 3ml aliquots and freeze dried.

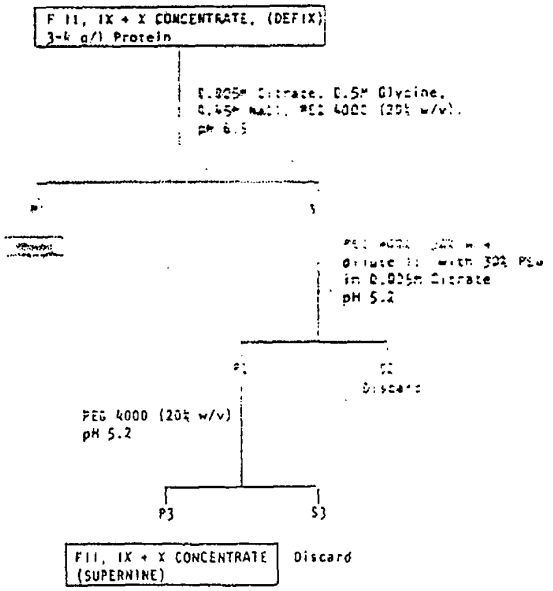

FIG 1

Scheme for the preparation of an advanced factor IX concentrate by polyethylene glycol fractionation.

TABLE 1

Two production batches of factor IX concentrate (Supernine), each prepared from 800 - 900 litres of fresh frozen plasma.

	Batch 100	Batch 101
Factor IX (u/ml)	108.0	71.0
Factor II (u/ml)	112.0	112.5
Factor X (u/ml)	85.0	125.0
Total Protein (g/l)	25.8	26.3
PEG 4000 (g/l)	4.4	6.6
NAPTT, 1/10 diln. (Seconds)	174.0	222.0
1/100	175.0	192.0
1/1000	194.0	207.0
Control	196.0	253.0
TGt50, unmodified (minutes)	>30	>30
Factor Xa generation (minutes)	30.0	35.5
Thrombin (u/ml)	0.002	0.004

RESULTS AND DISCUSSION

Supernine batches 100 & 101, prepared from 13 processed batches of DEFIX, are characterised in table 1. In comparison to DEFIX, factor IX concentration has been increased 2-3 fold and the specific activity 1-2 fold. Both batches satisfy the "in-house" quality control standards for thrombogenicity normally applied to DEFIX (i.e. NAPTT >150 seconds; TGt50 >10 minutes) and residual PEG is less than 1% (wt/vol) in the reconstituted product.

TABLE 2

NAPTT & NAPTR of fractions obtained by PEG processing. Preparations used for Supernine batches 100 and 101.

	DEFIX	P1	P3
NAPTT, 1/10 dilution, mean	154.3	116.1	174.6
standard deviation	17.3	16.6	22.7
NAPTR, 1/10 dilution, mean	0.71	0.59	0.82
standard deviation	0.08	0.08	0.12
Number of observations	13	12	13

The effect of processing on the distribution of potentially thrombogenic material can be more clearly seen by examination of the component batches used in the preparation of Supernine batches 100 and 101. By the NAPTT test (Table 2) there is a relative increase of thrombogenic material in the discard precipitate, P1, ($p < 0.001$) and a corresponding decrease in the product, P3, ($p < 0.001$). Similarly, fig 2 shows an increase in the NAPTT of the P3 product compared to the initial DEFIX material for almost all of the 85 batches processed. The same sample volume was used throughout the NAPTT test and the improvement following PEG processing is therefore particularly significant as the factor IX content of the P3 products is approximately 100 u/ml compared to 30 u/ml in DEFIX.

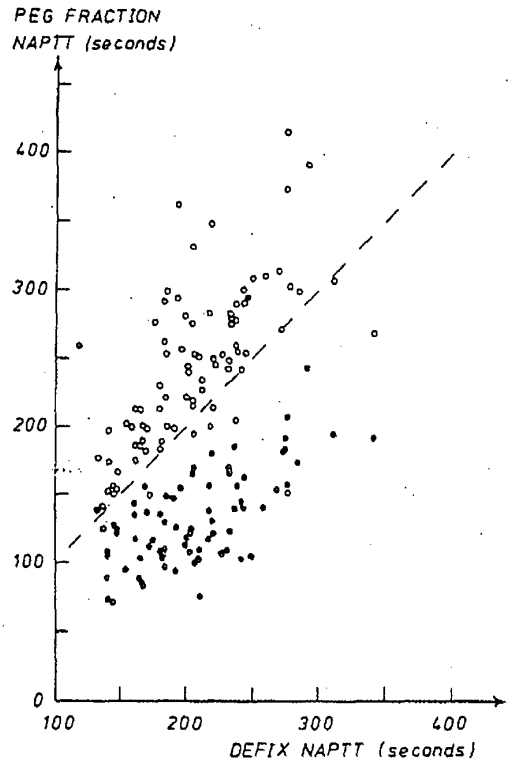

FIG 2

Change in NAPTT, 1/10 dilution, following polyethylene glycol fractionation of DEFIX.

0 - product fraction, P3
● - waste fraction, P1

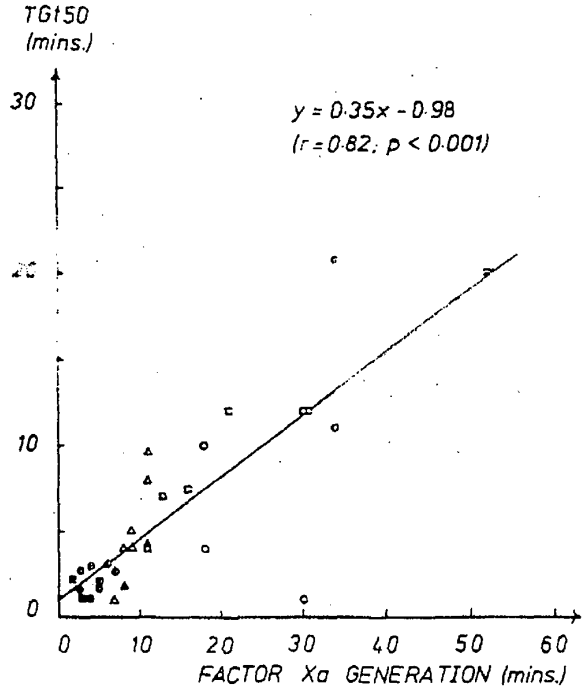

FIG 3

Correlation between the modified TGt50 and factor Xa generation tests.

Δ - DEFIX
○ - product fraction, P3
● - waste fraction, P3
□ - commercial concentrate
■ - FEIBA
▲ - NAPTT control plasma