0009

The Isolation Of FV111
Project Proposal

January 1976

S. Middleton.
R. Robson.
P. Foster.

1.0 INTRODUCTION

A report concerning factor VIII fractionation was submitted in November 1975 (1) That report contained some proposals for investigations that might be carried out in an attempt to increase the yield and purity of factor VIII. At that time the report was just discussed by Dr. A Johnston, Mr Watt, Mr Grant, Dr Foster, and Mrs Middleton, the most important areas for investigation were established and several further proposals were made.

It is now becoming apparent that insufficient supplies of fresh frozen plasma may increase our reliance on cryosupernatent fractionation. Development studies here might improve the yield and potency of this material. It is intended that this report should summarise proposals for future investigation, and it should be read in the context of the previous report.

2.0 PYROGENS.

2.1 DUMMY RUNS

These should be carried out using 0.02 M tris buffer which should pass through all stages of fractionation procedure, with strict adherance to time, temperature and pH of the normal process.

3.0 PLASMA

3.1 Length of Frozen Storage

Attempts should be made to fractionate plasma of the same frozen storage history in order to determine if there is an optimal frozen period with respect to final yield and purity of factor VIII. Dr. Johnston suggest that the plasma undergoes little change during two weeks to three months of storage but that changes do occur after this period.

3.2 Age

Attempts are being made to pool plasma of the same age

3.3/

3.3 Protective agents added to plasma prior to freezing

3.3.1 Polyethylene Glycol (PEG)

It has been suggested that the use of PEG may selectively enhance factor VIII recovery in the cryoprecipitate. The collection of plasma is already underway for initial exploratory experiments. (Appendix 1)

3.3.2 Heparin

Heparin added to the pre-frozen plasma should have a stabilising affect particularly in plasma that has been aged, plasma is being collected for initial exploratory experiments (Appendix) 1)

4.C FRACTIONATION

4.1 Thawing

4.11 Continuous removal of thawed plasma

Carefully controlled thawing at a high rate should produce more granular, less fibrous cryoprecipitate. Study will be carried out to allow the design of a continuous thawing system.

4.2 Extraction

4.2.1 Heparin

The presence of heparin (0.0.1 - 0.0.5 units per ml) added at the extraction stage, may improve the stability of the factor VIII. It may also reduce the solubility of the fibrinogen aggregates. (2)

4.2.2. pH

Lowering the extraction pH may lower the solubility of the fibrinogen. In order to achieve adequate buffering at the lower pH, it would be necessary to use a buffer with a lower pK valve that tris: The use of TES or HEPES has been proposed but as yet the in vivo toxicity of these buffers is unknown to PFC.

4.2.3. Assay Sample

It is considered that the addition of citrate is essential to stabilise this (TE_1) sample for assay. Citrate should be added to the assay sample only, to a final concentration of 0.02 M.

4.3 Alumina Adsorption

4.3.1. Gel Standardisation

It is proposed that a clinically rejected batch of F VIII be used (NY 130R) to prepare an experimental TE 1 batch. This material could be used for studies of the adsoptive properties of the alumina.

4.3.2. Double Adsorption

Dr. Johnston has suggested the alumina might remove aggregated material thus two adsoption steps should be investigated, an initial step for the removal of prothrombin complex, with a second step to remove aggregates. In the presence of heparin the importance of the initial adsoption would be reduced.

4.4 Purification of Intermediate Purity Factor VIII

4.4.1 Precipitation of Fibrinogen

It seems that a purification step used by other manufacturers for the removal of fibrinogen could be investigated. Lowering of the pH to 6.3. at $4^{o}C$ for a short time will allow precipitation of significant amounts of fibrinogen with only small losses of factor VIII reported (3)

4.4.2 Removal of Aggregated Material Using High Speed Centrifugation.

If the K rotor were available the use of a high speed centrifuge prior to filtration may reduce filtration losses due to the removal of aggregates. The feasability of such a step as part of a production process could only be assessed on experience with the rotor.

5.0 FRACTIONATION OF CRYOSUPERNATENT

Table 1 shows the data from some recent cryosupernatent fractionations. It is apparent that the material is of low potency and low specific activity.

5.1 INCREASE IN SPECIFIC ACTIVITY

Proposals outlined in previous sections, low extracting pH (4.22) or cold pH 6.3 precipitation (Section 4.4.1) are likely to increase the specific activity.

5.2 Ethanol Precipitation

A proposal has been made that 3% ethanol be used during cryoprecipitation to enhance factor VIII precipitation.

5.3 Potency

Extraction of the paste in a smaller volume of buffer should be considered, this will be limited by the apparent low specific activity of the paste. If possible the ionic strength of the buffer and added citrate should be reduced to allow for concentration after drying.

6.0 PEG FRACTIONATION

It is thought that the application of the PEG fractionation

steps should be reconsidered particularly with regard to the low potency cryosupernatent preparations. Table 2 shows previous data obtained during this procedure it can be seen that losses were mainly due to filtration.

7.0 FILTRATION

Pre Incubation Of Factor VIII at 30°C

7.1 Pre incubation at 30°C is at present being carried out prior to filtration.

7.2 Filtration at 30°C

An incubator is at present being designed to accommodate factor VIII and a 293mm filter in order to maintain both at 30-35°C during filtration.

7.3 Sampling

Samples should be taken at every stage of the process to determine where any losses are occurring.

7.4 Filtration losses due to aggregation on the filter

A recent publication (4) demostrates protein aggregates are retained by Millipore membrane filters. It is proposed that experiments be carried out to investigate the relative merits of the Gelman GA8 and Nuclieopore membranes which as the study described did not retain significant amounts of protein.

8.0. CONCLUSION

Figure I is an attempt to summarise proposed areas for investigation described in this report.

Summary of proposals for investigation

Protective agents added to prior to freezing

(1) Heparin
(2) PEG
(3) Haparin/PEG

THAWING

(1) Continuous feed from thawing tank.
(2) Ethanol addition during thawing (Cryosupernatant batches)

CRYO PRECIPITATE EXTRACTION

(1) Heparin addition
(2) pH

ALBUMIN ADSOPTION

Second adsorption stage

INTERMEDIATE PURIFICATION

(1) pH 6.3 + 4°C
(2) High speed centrifugation

PEG PRECIPITATION

FILTRATION Comparison of filtration properties of
Millipore
Gelman G A 8
Nucleopore.

SNB.007.0790

TABLE 1 DATA OBTAINED FROM FRACTIONATION OF CRYOSUPERNATENT And Fresh Frozen Plasma

Cryosupernatent

Run NO	Plasma Vol	Paste Weight g	g/l.c.s	Extract Vol L *	FV111 u/ml	Prot mg/ml	UFV111/mg prot
12	125	464	3.7	2.3	0.72	12.8	0.06
11	120	415	3.4	2.0	1.8	15.6	0.11
10	127	445	3.5	2.2	1.6	15.5	0.10
9	130	721	5.5	3.6	0.86	16.25	0.05
8	131	6655	5.0	3.3	1.36	15.7	0.08

Fresh Frozen Plasma

NY 136	125	1034 g	8.2	3.75 **	8.05	23.2	0.34
137	120	1178.5	9.8	3.9	6.0	17.8	0.3
138	124	843.5	6.8	3.72	6.65	15.2	0.4
139	125	1372.5	10.9	3.75	6.16	17.4	0.35
140	120	1039.5	8.7	3.6	10.0	18.1	0.55

* Extract volume 5ml Tris / g paste

** Extract volume 0.03 x plasma volume

PRSE0002225_0008

TABLE II

DATA OBTAINED FROM PEG FRACTIONATION OF FV111

	Intermediate Purity			P2			
Run No	%FV111 Recovered	u/ml FV111	u/mg prot	% FV111 Recovery	u/ml FV111	u/mg prot	Filtered % Recovery
23	32	2.3	0.12	25%	158	0.58	8%
24	23	1.41	0.12	23%	13.6	1.3	15.5%
25	23	1.39	0.07	20%	13.5	0.75	15 %
26	26	1.8	0.13	17.7%	11.9	0.82	16.5%
27	38	2.34	0.13	32%	19.95	1.1	13%
28	56	3.16	0.31	50%	28.	3.1	37%

REFERENCES

(1) Sarah Middleton Roy Robson
The present status of process operations for the isolation of FV111
internal Report November 1975.

(2) L. Thomas - 1950 Reference unknown communicated by Dr. Johnson.

(3) Personal communications C. Heldebrant.

(4) R.J. Hawker and Linda M. Hawker protein
losses during sterilising by filtration.
Lab. Practice. 24 12 805 1975

File fctr VIII 2.167
0010

Experiments to determine the effect of polymer addition to fresh frozen plasma on yield and purity of Factor VIII.

Plasma Requirements

8 x 10l plasma of the same age and collected within the shortest possible time to standardise frozen storage time.

Reagents

Supplied	10 vials solution A	(+ 2 extra)
	10 vials solution B	(+ 2 extra)
	10 vials solution C	(+ 2 extra)
	10 vials solution D	(+ 2 extra)

Method

20l plasma can be made up with each solution. Solutions have been prepared so that the contents of one vial can be added directly to a 2l plasma pool, the contents of the vial should be added with thorough mixing.