METHODS FOR PREPARING NON-INFECTIVE BLOOD PRODUCTS

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PRODUCTS FROM PLASMA FRACTIONATION

STANDARD PRODUCTS		SOME POTENTIAL NEW PRODUCTS
LOW RISK	HIGH RISK	
ALBUMIN	FIBRINOGEN	ANTITHROMBIN III
PLASMA PROTEIN SOLUTION	FACTOR VIII	FACTOR VII
IMMUNOGLOBULIN	FACTOR IX	FIBRONECTIN
		PLASMINOGEN
		γ-1 ANTITRYPSIN
		C-1 INACTIVATOR
		CAERULOPLASMIN
		TRANSFERRIN
		FACTOR XIII
		THROMBIN
		PROTEIN C

HEPATITIS INFECTIVITY STUDIES (1951-1954)

(from Barker et al. Am. J. Med. Sci. 263, 27-33, 1971)

SOLUTION	NUMBER OF PRISON VOLUNTEERS		
	TOTAL	CLINICAL HEPATITIS	HAA IN SERUM
PLASMA, 0 DIL	37	23	25
10^{-3} DIL	5	2	3
10^{-4} DIL	5	1	3
10^{-5} DIL	5	0	2
10^{-6}	5	0	3
10^{-7}	5	0	2
ALBUMIN (100 ml)	5	2	2
SPPS	5	2	2
ALBUMIN (60^{0}, 10hr)	15	0	0
PLASMA (60^{0}C, 2hr)		4	4
(60^{0}C, 4hr)		3	3
BETAPROPIOLACTONE		5	4
UV IRRADIATION		22	22

HEPATITIS RISK FROM IMMUNOGLOBULIN

PREPARATIVE METHOD	INFECTIVITY
1. FROM SPIKED POOL	
1.1 COLD-ETHANOL FRACTIONATION	NON-INFECTIVE IN VOLUNTEERS (1)
1.2 ZINC FRACTIONATION	INFECTIVE IN VOLUNTEERS (2)
1.3 ION EXCHANGE CHROMATOGRAPHY	INFECTIVE IN CHIMPS (3)
1. FROM NORMAL POOLS	
1.1 COLD ETHANOL	PRODUCT HB_SAg -ve (4)
1.2 AMMONIUM SULPHATE FRACTIONATION	PRODUCT HB_SAg +ve (4)

REFERENCES

1. Murray et al Proc Soc Exp Biol Med 83, 554-555, 1953.
2. Barker et al Am J Med Sci 263, 27-33, 1971.
3. Zolton et al ISBT Congress Abstr M-327, 1982.
4. Hoofnagle et al Transfusion 15, 408-413 1975.

SOME PROPOSED SOLUTIONS TO THE HEPATITIS PROBLEM

1. DONOR SCREENING (for HB markers)
2. PRODUCT SCREENING (for HB markers)
3. VACCINE (HB)

PROBLEMS

1. SCREENING NOT SENSITIVE ENOUGH
2. POOR CORRELATION BETWEEN MARKERS AND INFECTIVITY
3. NANB
4. ? OTHER INFECTIOUS AGENTS (CMV, AIDS)

POTENTIAL METHODS STUDIED

METHOD	ORGANISATION
1. ACCREDITED DONORS	KABI, 1974 (Thromb Res 5, 439-452)
2. REMOVAL OF VIRUS	
2.1 SPECIFIC (HB)	
2.1.1 adsorption (virus)	KABI, 1981 (J. Virol meth 3, 213-228
2.1.2 adsorption (product)	Speywood (unpublished) Ortho, 1982 (ISBT Abstr M327)
2.2 NON-SPECIFIC	
PEG PPTN	NYBC/PFC, 1976 (J. Lab Clin Med 88, 9
3. INACTIVATION OF VIRUS	
3.1 SPECIFIC (HB)	
addition of anti-HB IgG	BoB, 1980 (Lancet II 68-70) NRC, 1983 (Vox Sang, in press)
3.2 NON-SPECIFIC	
3.2.1 Chemical. BPL/UV	Biotest, 1980 (Thromb. Haemost. 44, 138-142)
detergent	Shanbrom, 1982 (US Patent 4, 314, 997
?	Immuno, 1983 (unpublished)
3.2.2 Heat	Abbot, 1977 (Thromb. Haemost. 38, 201) ARC, 1981 (J. Biol. Chem. 256, 12140-47 Behringwerke, 1979 (GERMAN PATENT 291671

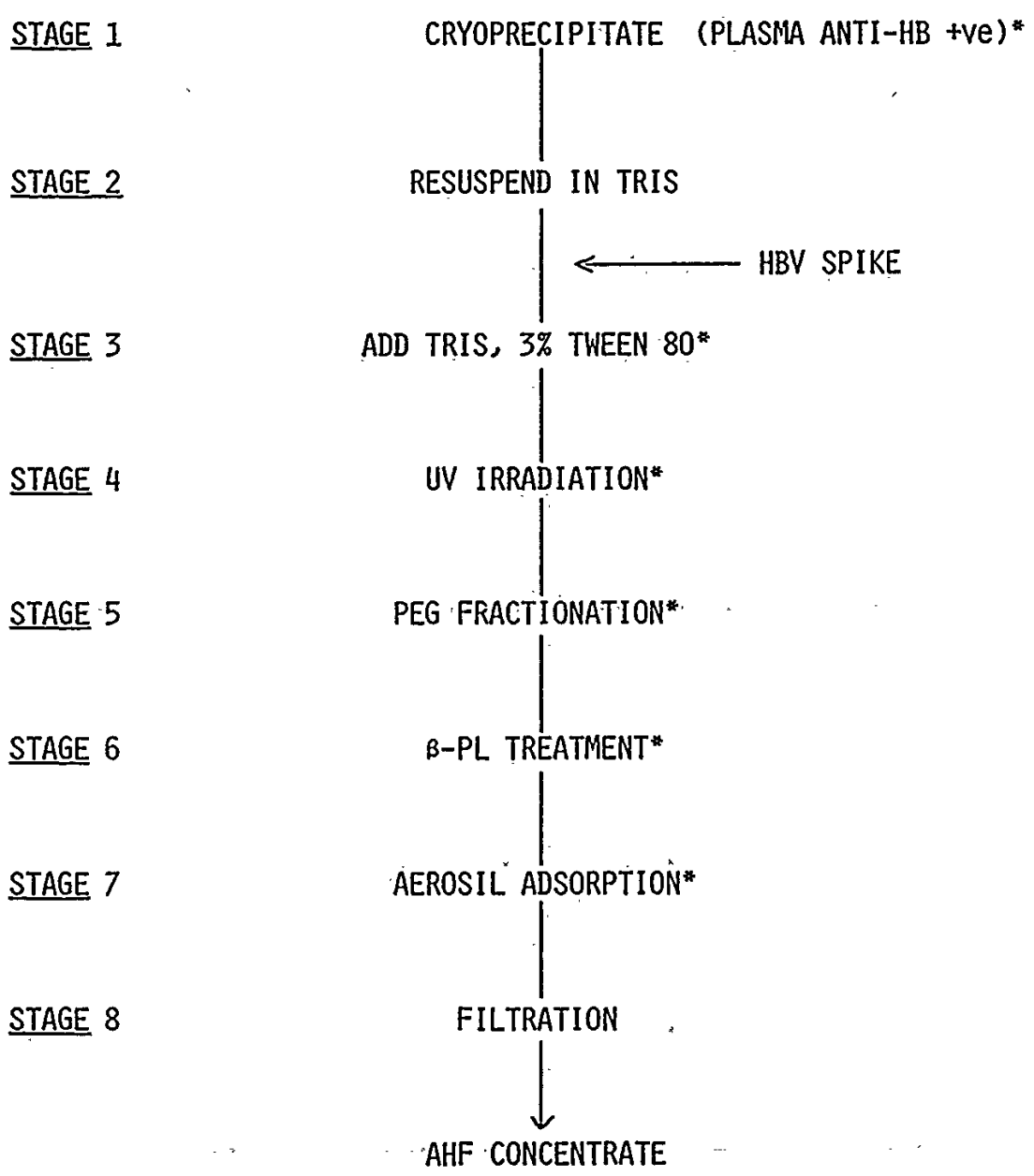
BPL/UV TREATMENT WITH SPIKED PLASMA

USE			COMMENT
1.	BPL OR UV	(1)	INFECTIOUS
2.	COMBINED		
	2.1 FIX	(2)	1/4 CHIMPS INFECTED (inactivated 50% FIX, 60% FII)
	2.2 FVIII	(3)	0/2 CHIMPS INFECTED (? mechanism, ? yield)

REFERENCES

1. Barker et al Am J Med Sci 263, 27-33, 1971.
2. Prince et al Thromb. Haemost. 44, 138-142, 1980.
3. Stephan et al ISBT Congress Abst. Th 134, 1982.

BIOTEST METHODS FOR BPL/UV TREATMENT OF FVIII



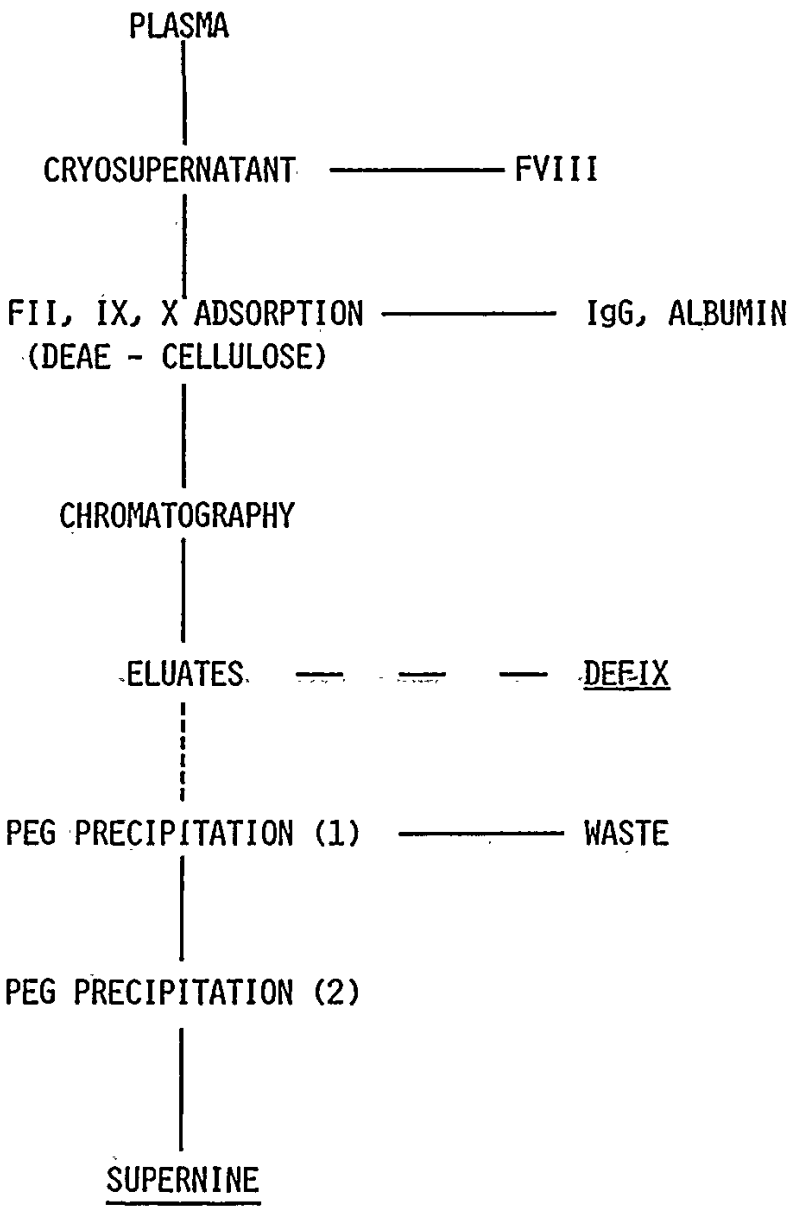
* COULD CONTRIBUTE TO REMOVAL OF INFECTIVITY.

CHEMICAL METHODS

POTENTIAL PROBLEMS

1. PROOF OF NON-INFECTIVITY
2. TOXICITY
3. FORMATION OF NEO-ANTIGENS
4. YIELD

PFC PROCESS FOR FIX CONCENTRATE



SUPERNINE PROJECT

1970 PROJECT COMMENCED

PARTICIPANTS:

PFC (J. K. SMITH & S. M. MIDDLETON)
NEW YORK UNIVERSITY (A. J. JOHNSON)

AIM

TO REPLACE DEFIX WITH A CONCENTRATE 3-5 TIMES MORE POTENT AND WITH REDUCED HBsAG CONTENT.

APPROACH

USE PEG 4000 TO REMOVE HBsAG FROM DEFIX AT pH REMOTE FROM THAT OF FIX IEP, THEN PRECIPITATE FIX AT ISOELECTRIC POINT. OPTIMISE RECOVERY OF FIX AND REMOVAL OF HBsAG BY MANIPULATION OF pH, PROTEIN CONCENTRATION, IONIC STRENGTH, PEG CONCENTRATION.

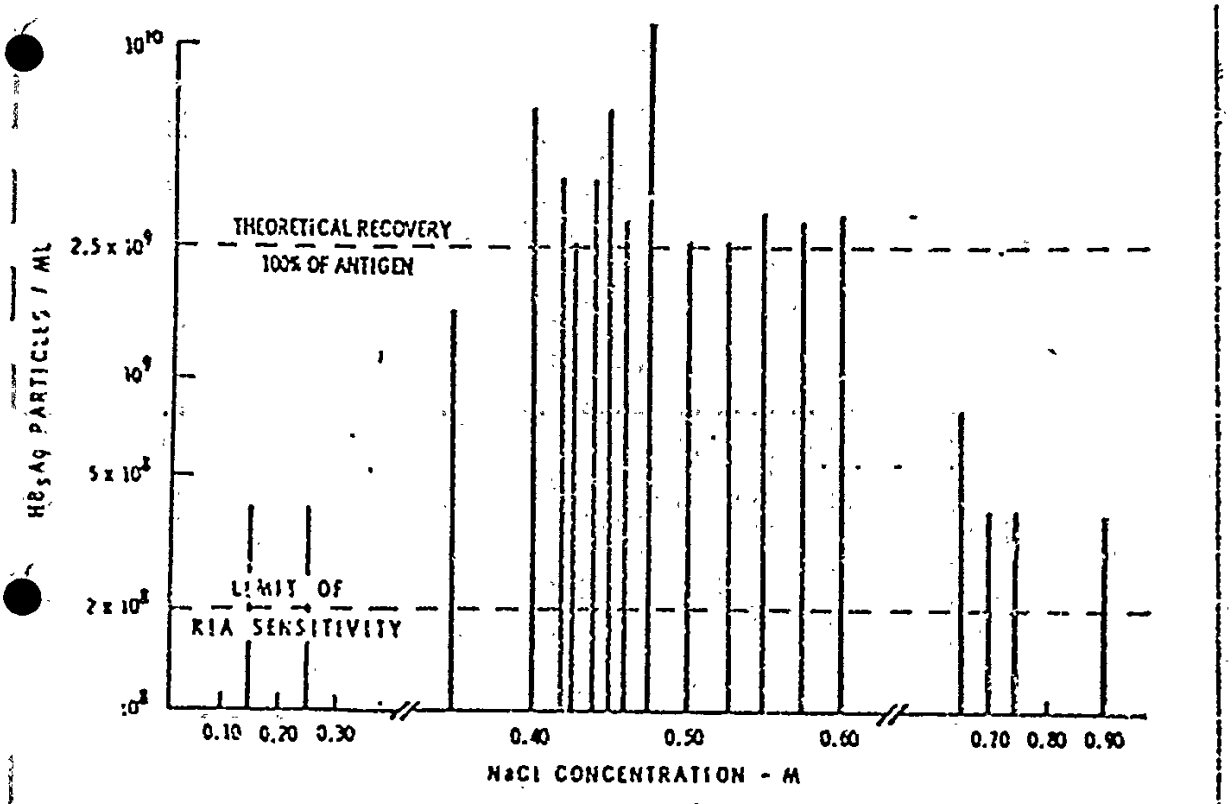
1973 MARK I PRODUCT USED IN CLINICAL TRIALS

MARK II DEVELOPMENT INITIATED

1976 MARK II CHEMISTRY FINALISED

SCALED-UP PRODUCTION INITIATED

1977 ROUTINE PRODUCTION - USING FAILED DEFIX LOTS


THEORETICAL RECOVERY
100% OF ANTIGEN
LIMIT OF
RIA SENSITIVITY
HBsAg PARTICLES / ML
NaCl CONCENTRATION - M
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90

CONCENTRATION AND RECOVERY OF HBsAG BY PRECIPITATION* FROM A MIXTURE OF CONTAMINATED SERUM AND II, (VII), IX AND X CONCENTRATE

ORIGINAL HBsAG LEVEL †---	HBsAG -- (P-1)		II, (VII), IX & X CONC. (S-1)	
PART./ML.	X CONC.	PART./ML	X CONC.	PART./ML.
2×10^{10}	100	1.6×10^{12}	-	-
5×10^{8}	500	4.0×10^{11}	1,000	NEG
5×10^{7}	200	1.28×10^{10}	200	NEG
5×10^{6}	2,000	9.0×10^{9}	200	NEG
5×10^{5}	3,000	1.5×10^{9}	-	-
5×10^{4}	20,000	1.12×10^{9}	-	-

* THE ANTIGEN WAS PRECIPITATED WITH 20 PER CENT PEG (W/V), 0.45M NACL.

† THE HBsAG WAS BROUGHT TO THE ORIGINAL LEVELS BY ADDING SERUM CONTAINING KNOWN AMOUNTS OF ANTIGEN TO II, (VII), IX AND X CONCENTRATE. THE ANTIGEN LEVELS IN P-1 AND S-1 WERE DETERMINED BY AUSRIA II RIA.

EFFECT OF FILTER PORE SIZE ON RETENTION OF HBsAG DELIBERATELY ADDED TO ALBUMIN AND AGGREGATED BY PEG.

FILTER* PORE SIZE (MICRON)	0.22	0.45	0.65	0.80	1.0	1.2	3.0	7.0	14.0
% YIELD HBsAG IN FILTRATE †	0	0	1	1	65	50	63	100	100

* MILLIPORE.

† THE FILTRATE WAS ASSAYED AT ITS ORIGINAL VOLUME BY CEP, RPHA, AND AUSRIA I RIA.

ADVANTAGES OF SUPERNINE

1. INCREASED POTENCY OF FACTORS II, IX & X

2. REDUCED INFUSION VOLUME

3. REDUCED RISK OF SIDE EFFECTS/ADVERSE REACTIONS

   3.1 HEPATITIS

   3.2 THROMBOGENICITY

   3.3 ALLERGIC REACTIONS

   3.4 NO FVIII:CAG

HEAT TREATMENT (60°C, 10hr)

STABILISER	PRODUCT
1. CAPRYLATE TRYPTOPHANATE MANDALATE	ALBUMIN/SPPS
2. CITRATE	AT III
3. LIGANDS HEPARIN LYSINE	 AT III PLASMINOGEN
4. CARBOHYDRATES /AMINO ACIDS	FII (Behringwerke patent) FVIII F XIII AT III PLG FIBRONECTIN (Cutter) FIX etc (PFC, unpublished)

PASTEURISATION PROBLEMS

1. CONCENTRATION OF VIRUS

   - INACTIVATES 10^4 CID/ML (PRINCE, NYBC)

2. PROTECTION OF VIRUS BY PROTEIN STABILISER.

3. NANB .

4. CROSS CONTAMINATION AFTER PASTEURISING

BEHRINGWERKE PROCESS (FVIII)

FVIII YIELD 8% (HEIMBURGER ET AL. HAEMOSTASIS 10 (SI) 204, 1981)

REASONS:

(i) NEEDS HIGH PURITY PRODUCT.

(ii) PASTEURISATION LOSS OF 50%

HYLAND METHOD (FVIII)

1. ? Heat Process ? Tempr ? Time

2. Plasma Spike

   30 000 CID ($10^{4.5}$) HB infection (2 Chimps)

   300 CID ($10^{2.5}$) 2 Chimps HBsAg +ve, 7½ - 9 months.

3. ? Yield

NOTE: Recommended HB spike = $10^{3.5}$ {Tabor et al. Thromb. Res. 22, 233-238, 1982}

REFERENCES:

1. HOLLINGER et al. M.V. Pett Symp. on Viral Hep. (Abstr) 1982.

2. DOLANA et al. Clin. Res. 30, 722 1982.

TO IMPROVE FVIII YIELD

NEEDS

(i) NEW FRACTIONATION PROCESS TO ACHIEVE APPROPRIATE QUALITY WITH GOOD YIELD.

(ii) BETTER STABILISATION OF FVIII DURING PASTEURISATION.

HIGH PURITY METHODS

STANDARD

1 PEG PRECIPITATION (JOHNSON)

2 COLD-ACID PRECIPITATION (CUTTER, ALPHA)

3 PEG-GLYCINE PPTN (HYLAND)

NEW

1 POLY ELECTROLYTES (JOHNSON, SPEYWOOD)

2 HEPARIN - CIG PPT (ROCK)

3 METAL-ION PPTN

4 ASSISTED - ACID PPTN

PFC RESEARCH

PROCEDURE	COMMENTS
1. FVIII	
ZINC FRACTIONATION	REMOVES 80% FIBRINOGEN 70% FIBRONECTIN RETAINS 90% FVIII 90% VWF
PASTEURISATION WITH NEW STABILISER	20% FVIII LOSS
2. FIX	
PASTEURISATION WITH NEW STABILISER	35% FIX LOSS NO CHANGE IN IN-VITRO THROMBOGENICITY

PFC PROGRAMME

1. FIX CONCENTRATES

1.1 SUPERNINE UNDER CLINICAL EVALUATION.

1.2 PASTEURISED CONCENTRATES TO BE PREPARED FOR THROMBOGENICITY TESTING.

2. FVIII CONCENTRATES

2.1 PASTEURISED CONCENTRATE FOR CLINICAL EVALUATION.

3. OTHER PRODUCTS

3.1 PASTEURISED AT III TO BE PREPARED FOR CLINICAL EVALUATION.

3.2 PASTEURISATION TO BE APPLIED TO

- 3.2.1 FVII
- 3.2.2 FIBRONECTIN
- 3.2.3 IgG
- 3.2.4 Etc Etc