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1. INTRODUCTION

This report should be read in conjunction with the book of abstracts. Presentations were either plenary lectures (prefix PL), symposia (prefix S) or posters (prefixed by day of presentation, e.g. M). Items in this report have been organised according to topic rather than any particular session or day.

2. THE PREPARATION OF NON-INFECTIVE BLOOD DERIVATIVES

2.1 Factor VIII

Two new products were introduced at the Congress, one from Biotest and one from Hyland, there was no presentation from Beringwerke but literature was available at the Trade Exhibition giving details of animal experiments etc.

2.1.1 Biotest Concentrate

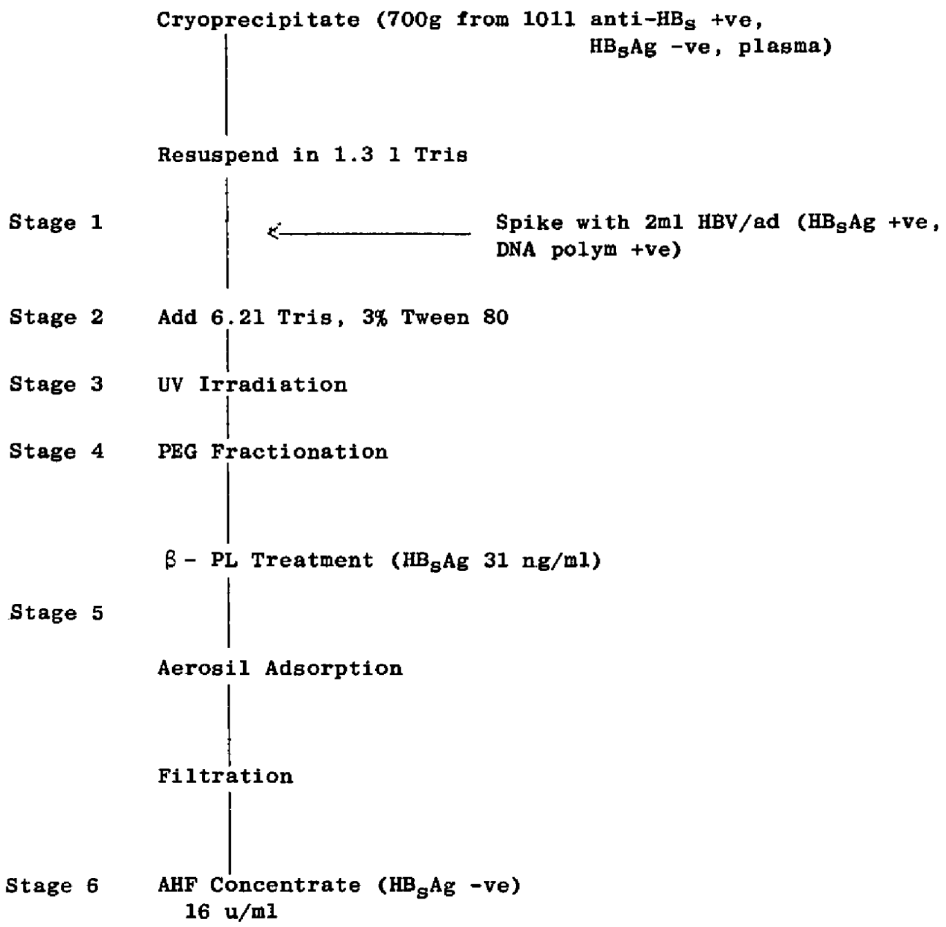
This was dealt with in symposia (S-23-5, S-23-6, S-23-7) and a poster presentation (Th 134). In presenting chimpanzee data Prince (S-23-7) argued that virus dose correlated with incubation period and that with a known starting titre the degree of inactivation could therefore be measured. The following methods were said to give a progressive increase in inactivation according to increasing incubation period.

Method	HB Reduction
Pasteurisation	10^4
β-PL/UV	$10^{6.9}$
β-PL/UV + Aerosil	$10^{8.3}$

with β-PL/UV alone using spiked plasma (1 part per 1000) half of the test animals were infected and Aerosil (fumed silica) adsorption was required, this was said to adsorb 95% of the surface antigen. Following the final β- PL/UV + Aerosil scheme, both control chimps developed non-A, non-B hepatitis with incubation period up to 25 weeks.

In describing the detailed method Stephan (S-23-6, Th-134) said that FVIII was very sensitive to β- PL treatment and that the conditions had to be modified from those used previously (eg FIX), though the same UV apparatus (thin film, rotating cylinder) was used.

The manufacturing method was given in Th-134 as:-



Material at stage 1 infected 2 chimpanzees while material from stage 5 was non-infective in 2 chimpanzees

2.1.2 Hyland Concentrate

This topic was not listed in the programme but Dr. Dolan was invited to present a report of the work following S-23-7 by Prince. The method was said to involve pasteurisation and details of chimpanzee experiments were presented.

Concentrate was spiked with hepatitis B and heat treated. Control chimps developed hepatitis (ALT peak) after 6 weeks, ALT levels then returned to normal but were raised again at week 11. This was thought to be evidence of infection by both B and non-A, non-B. The experimental chimps have shown no evidence of infection to date (ie after 8 months) but they are still being observed. It was also stated that the viral challenge that had been inactivated in this study was 300 CID (Chimpanzee Infective Doses) but that a higher challenge (30000 CID) had not been "completely" inactivated by pasteurisation. No details of this experiment were given.

The method of stabilisation of FVIII was not reported but privately it was admitted that this had been discovered accidentally and that the yield was about 200 iu/l. The method is therefore probably different to the sugar/glycine stabilisation of Beringwerke.

2.1.3 Other Studies

Rubenstein (F-89, F-90, F-110) reported studies of heat treatment applied to coagulation factor concentrates in freeze dried form. Products from different manufacturers behaved differently. One product would not withstand heat treatment while another gave 50% retention of FVIII:C after 10 hours at 80°C or 71% retention of FVIII:C after 1/2 hour at 100°C.

Chimpanzee studies are underway to establish the heating conditions necessary to inactivate hepatitis virus(es) in freeze dried concentrates.

2.2 FIX Concentrates

The only studies involving FIX were those of Rubenstein (F-89, F-90) in which a FIX concentrate in the freeze dried state was heated for 10 hours at 100°C without loss of activity. As with FVIII, the chimp data is pending.

The Biotest β - PL/UV process was briefly mentioned (S-23-6) and the published 50% loss of FIX activity was confirmed. Previously published data concerning "spiked" experiments showed that 1 of 4 chimpanzees was infected (Thromb. Haemost. 44, 138-142, 1980). No further data on "spiked" experiments were produced. Literature at the Trade Exhibition contained extensive results of chimpanzee experiments etc but this appears to be from "routine" batches which had not been "spiked" with a defined titre of virus. This literature did not mention the results of the spiked experiment. FII was reported to lose 40% of its activity after βPL/UV treatment (F-134) and showed different ion-exchange adsorption characteristics

2.3 Fibrinogen

Again data on heat treatment and β - PL/UV were reported.

2.3.1 Rubenstein (F-110) showed the following recoveries after heating 2 products in the freeze dried state.

A. 1 gram, 77% protein, 71% clottable, 40% citrate.
B. 1 gram, 90% clottable, 0.4g NaCl, 1.2g citrate.

Product	Heating	% Recovery
A	10 hrs, 60°C	27
	11 hrs, 60°C	46
	17 hrs, 60°C	27
	11 hrs, 65°C	4
	11 hrs, 65°C	11
B	10 hrs, 60°C	100
	10 hrs, 65°C	100
	23 hrs, 65°C	100
	3 hrs, 100°C	74
	3 hrs, 123°C	13

All product A experiments resulted in poor solubility while the final product B experiment also showed reduced solubility. As with FVIII & FIX, chimp data is still awaited.

2.3.2 Biotest presented β - PL/UV treatment (F-134) applied to the final product (Kabi fibrinogen) or to cryoprecipitate supernatant (S-23-5) from which fibrinogen was then prepared.

The properties of the 2 resulting products were compared. The product treated in its final form was no longer coaguable while that prepared from "sterilised" feedstock retained its activity.

Details of the methods given were:-

Modified fibrinogen (Kabi) - Ig fibrinogen resuspended in 100 ml H_2O. β - PL added (0.5mg per mg fibrinogen at pH 7.2.)

Biotest Method

(i) β - PL Treatment - Freshly distilled β - PL added dropwise at room temperature to citrated plasma pool (sic) - probably cryosuper from citrated plasma. β - PL concentration was 0.05 to 0.8 vol % and pH was held at 7.2 using 1M NaOH. (Ref Stephan. Z. Clin. Chem. Clin. Biochem, 7, 518, 1969).

(ii) UV-Irradiation - used 2 watts/cm^2 at 256 nM.

(iii) Fibrinogen Prepn. - Aerosil (150 mg/g protein was added to β - PL/UV treated plasma. The Aerosil was collected by centrifugation after stirring for 2 hrs. Fibrinogen was eluted at pH 9.5 using a solution of 15% NaCl, 30mM citrate and 2000 K1 units/ml Trasylol (at S-23-5 elution from Aerosil was said to be by 20% Xylene at pH9). The eluate was dialysed against 30mM citrate for 16 hours at pH 7.2.

No animal studies were reported.

2.4 Immunoglobulin

The problem of hepatitis contamination of immune globulin was studied in a presentation by Ortho (M-327). The poster showed that IgG prepared from plasma "spiked" with BoB titred HBV was RIA negative, using ion-exchange chromatography as the preparative method. The published abstract mentions that this product has been tested in chimpanzees "with preliminary results suggesting that the safety question for HBV has been satisfied". However, at the poster the author conceded that all chimps (1 control and 2 experimental) have now developed hepatitis. He thought that this may have been due to a poor lot of ion-exchange resin (Pharmacia) and indicated that they intended to repeat the study.

2.5 Personal Comments on Methods to Resolve the Hepatitis Problem

2.5.1 β - PL/UV Methods

In the process presented for FVIII the mechanism(s) of viral inactivation/removal are difficult to discern. There would appear to be 6 possible components with only their combined effectiveness tested in the animal study. These components are:-

(i) HB Antibody in the Plasma Pool - The titre of this was not stated but it seems to have been insufficient alone to protect the control chimp. (i.e. tested with spiked cryoppt extract).

It is of interest to note that in his lecture on fractionation Brummelhuis (PL-1-8) gave a figure of 0.4 iu/ml IgG(H) to be added to a product such as factor VIII for HB protection.

Presumably non-A, non-B protection may be provided by IgG(N) added to the product, or perhaps administered separately (intravenously)?

(ii) Tween 80 - This acts by stripping off the viral coat, a mechanism currently being investigated by the Scottish group. No details of detergent fractionation were given.

(iii) UV-Irradiation - Conditions not specified but probably the same as the fibrinogen method (2.3.2 above).

(iv) PEG Fractionation - Details not given but could conceivably involve a 3.5% cut which could precipitate some virus.

(v) β - PL - Conditions not given, but certainly more gentle than previous examples.

(vi) Aerosil Adsorption - said to be essential to obtain non-infective product. Exact conditions not given.

Aerosil is an effective adsorbent of fibrinogen and at one time formed the basis of a high-purity method being researched at NYBC. The method was said to be finally abandoned due to problems of activation, etc (Horowitz, personal communication). The use of Trasylol in the fibrinogen method (2.3.2) may be relevant here, though no mention of this was made for the FVIII process.

Although FVIII was said to be very sensitive to β - PL no information was given on yield, degree of inactivation etc. Hence a considerable number of important questions remain to be answered.

2.5.2 Heat Treatment

The work of Rubenstein using labile factors in their freeze dried state is very interesting but freeze drying is also likely to protect the virus and infectivity data is essential.

The Hyland product is perhaps the most interesting. If the yield indicated (200 iu/l) is confirmed this is probably higher than the present method of manufacture for Hemofil and therefore represents a definite break-through in FVIII stabilisation. Will this ever be published?

3. FACTOR VIII STABILITY

3.1/

3.1 Stability in Fresh Plasma

Rock (F-78) presented data on the addition of protease inhibitors and calcium to plasma. Her results were:-

(i) The addition of benzamidine (1mM), heparin (2 u/ml), DFP (5mM), SBTI (0.1 mg/ml), aprotinin (100 K1 u/ml), PMSF (1mM) to CPD plasma gave no protection over 24 hrs at 22°C.

(ii) 24 hour stability was significantly improved at higher concentrations of DFP (5-10 mM) and to a lesser extent by increased heparin concentration (4-8 u/ml). Increase in PMSF (2-3 mM) gave less improvement than heparin. Benzamidine was not effective.

(iii) Activation and subsequent degradation of FVIII:C by added thrombin was not prevented by DFP (10 mM) or Benzamidine (1 mM). In contrast heparin (8 u/ml) completely prevented thrombin activation and degradation of VIII:C.

(iv) For collection in heparin, CPD + heparin and CPD, VIII:C was completely stable for 24 hours if physiological levels of ionised calcium were maintained in the presence of an effective anti-coagulant.

For plasma in CPD or CPD + heparin, loss of activity could not be prevented.

(v) When fresh CPD plasma was recalcified to 4 MG% ionised calcium the subsequent VIII:C activity was equivalent to that of heparin plasma.

(vi) For blood collected in heparin with decreasing concentrations of CPD the VIII:C activity increased progressively to that observed with heparin alone.

(vii) Recalcification of CPD + heparin plasma from 0-4 hours after collection was effective in restoring VIII:C, the restored activity was stable. If recalcification was carried out at 6 hours, only partial recovery was achieved and the recovered activity was not stable.

Recalcification at > 6 hours gave a further reduction in the degree of recovery and greater instability.

3.2 Stability of Fresh Frozen Plasma

McLellan (F-117) studied the stability of VIII:C (2-stage assay) and VIIIC:Ag (agarose gel diffusion) in plasma samples stored at -35°C (ie -40°C freezer).

For 100 samples, comparison of fresh values versus stored (1 month) showed no loss of VIII:C activity.

i.e. linear correlation $y = 0.98X + 1.26$

$r = 0.97, p < 0.01$

For VIII:C at time 0 (X) verses VIIIC:Ag at 1 month (Y) the correlation was:- $Y = 0.9X + 2.09$

$r = 0.9$

In discussing the study McLellan said that progressive loss of VIII:C was evident from about the 3 month point at -35°C. He believed that the storage temperature should be between -30°C and -50°C (with signs of activation below -50°C). He had also found group O plasma to have significantly lower levels of VIIIC:Ag than group A.

Biotest (F-134) gave their specification of fresh plasma as plasma frozen to -40°C within 2 hours of collection by plasmapheresis and stored at -40°C for not more than 2 weeks. Plasma outside this definition (eg held at -20°C for 4 weeks) was categorised as "stored plasma".

3.3 Stability of FVIII Concentrates

Levin et al (F-118) reported the continuous intravenous infusion of FVIII concentrate. 15 - 20% greater increments in FVIII activity per unit FVIII administered were achieved compared to bolus therapy. The crucial factor was the availability of a new concentrate which was stable in solution for a number of hours at room temperature. Armour concentrate (Factorate Generation II) was found to be stable after 12 hours at room temperature. Infusions were carried out successfully in 12 hour sessions for periods of 5 days (7800 iu) up to 17 days (36900 iu).

In establishing secondary standards for FVIII:C, Panicucci et al (F-86) reported that their intermediate purity concentrate standard degraded at 1.1% per year at -25°C while the plasma standard gave a predicted loss of 4.85% per year at -25°C.

4. OTHER FVIII PRESENTATIONS

In discussing the problem of achieving self-sufficiency from an all voluntary blood donor programme Cash (S-22-3) gave details of the SNBTS programme to meet clinical demands into the 21st Century. While Davey (S-22-4) argued that treatment should be restricted to avoid red cell wastage and the necessity for plasmapheresis. In discussing the possible role of "hepatitis-free" concentrates it was generally agreed that these should be restricted to use in haemophiliacs with no previous signs of infection.

The use of FIX concentrates in FVIII inhibitor patients was reviewed. Lusher (S-23-2) noted that a random double blind trial of Autoplex Vs Proplex will be completed in September 1982 and speculated that the active ingredient was "IXa, Xa, VIIa, something else or a combination of these". Seghatchian (S-23-3) favoured activated protein C, activated VIII, or activated VIIa (sic). So far he has been unable to separate activated protein C from VIIa. Hewitt et al (F-122) reported the development of an inhibitor to porcine FVIII in a patient who already had an inhibitor to human FVIII. Bleeding was eventually controlled with FEIBA.

The preparation of a Factor VIII Concentrate by chromatography (methylated trisacryl) was described (F-81). The scheme was:-

Cryoprecipitate

|

Solution in buffer 1 (0.02M tris - glycine, pH 5.7)

|

Adsorption

|

Wash 1 (buffer 1)

|

Wash 2 (buffer 1 + 0.15M NaCl, pH 5.7)

|

Wash 3 (0.02M tris - glycine, pH 7.2)

|

Elution (0.02M tris - glycine + 0.5M NaCl, pH 7.2)

|

FVIII:C

Results.

	% VIII:C Recovery	Specific Activity (iu/mg)	Protein (mg)
Cryoprecipitate	(100)	0.33	333
Wash 1	1.9	0.02	91
Wash 2	9.1	0.06	170
Wash 3	0	-	19
Eluate	90	1.86	53

From the total protein data (ie 333 mg as Cryo) the process scale would seem to be about 300 ml plasma. A second scheme using sodium acetate and diethylmalonylurea gave only a 77% yield.

Prowse (F-83) reported a comparison of FVIII yield from Group A versus Group O donors for thaw-siphon cryoprecipitate. The results were:-

	Group A	Group O
Total plasma FVIII:C u/570ml	565	485
Total cryoppt FVIII:C u/90ml	451	338
% yield	80	70

Prowse (F-92) also reported the routine use of thaw-siphon cryoprecipitate. Products were prepared by pooling 3 donations and, with 1.5 technicians, 166 pools could be processed per month. 1/16th of the product was sacrificed for quality control testing. The clinical results were:-

	Thaw-siphon Cryo		Freeze Dried Cryo
	Group A	Group O	
In vivo recovery (%)	115	92	121
1/2 life (hrs)	9.7	9.3	9.8

5. IMMUNOGLOBULINS

5.1 Preparation of IgG for Intravenous Use

Methods presented for preparing I.V. IgG included the use of β - PL (Th-185) and ion-exchange chromatography (Th-135, Th-136, Th-253).

Nydegger (S-19-2) reviewed the characteristics of various preparations. He indicated that an ideal product would be native and free of anti-complementary activity and PKA. It should have proper in vivo potency including platelet binding properties (see B. J. Haematol, in press) and normal sub-class composition. The importance of IgG3 was stressed (see Beck in Clin. Expt. Haem.) this was particularly important for Rubella, Polio 1, II & III, and Herpes.

Data on CIq binding was given:-

Product	ACA/(C150/mg)	% CIq Bound
Untreated IgG	600	18
Enzyme	<20	0 - 5
Sulphonated	35	82
PEG/HES	300	9
PEG	atypical	0 - 7
Adsorption/pH4	<20	0 - 2
β - PL reduced	<20	2 - 3

The correlation of anti-C values with side effects was queried as there have been instances of hypertension when anti-C levels were low. Nydegger agreed that anti-C was not the only property relevant. The clear inference from Nydegger's review was that the most "native" product was best and that the pH4 method comes closest to this so far.

Stephan (Th-185) presented results using β - PL treatment to remove anti-C activity which showed a 7S molecule (compared to pH4 (7S), pepsin (5S), papain (3.5S)). The efficacy of various products was studied in a mouse protection test using Pseudononas, Salmonella, Influenza A and Tetanus toxin. The 7S preparations were more effective than those that were fragmented.

Suomela (TH-135) gave details of a chromatographic method for IV IgG. The method was:-

Plasma

↓

Aerosil adsorption I (to remove lipids & proenzymes)

↓

Gel filtration (Sephadex G-25 to lower ionic strength)

↓

Aerosil adsorption II (to remove IgM)

↓

DEAE-Seph CL-6B (20mM phosphate, pH 7.0)

↓

Elute IgG

↓

Adjust pH to 5.8

↓

SP-Sephadex C-50 (IgG binds up to 100mg protein/ml gel)

↓

Elute IgG with glycine buffer

↓

Add albumin as stabiliser

↓

Sterile filter & freeze dry

Typical production data was:-

Stage	Volume (l)	Protein (g/l)	Total IgG (g)	IgG Yield (%)
Plasma	63.2	57.3	526	100
Aerosil I	57.3	53.9	400	77
Seph-G25				
Aerosil II	78.5	33.7	370	70
DE-Seph	103X	3.3	320	65
SP-Seph	12.2X	22.5	274	53

Product characteristics were:-

IgG (7S)	99% (by CAE)
Polymers	<0.5%
Dimer	<0.5%
PKA	<5%
IgA/IgM	Not detectable
Prekallikrein	0
Kallikrein	0
Plasmin/plasminogen	Very low
ACA	Low

Data was also presented by Suomela (Th-187) on the antibody composition of the product and on the sub-class distribution. Sub-class distribution was found to be similar to the starting plasma and antibodies against Rubella, CMV, Influenza A, Herpes Simplex, Mycoplasma, Toxoplasma and Tetanus were all present with a yield of 55-65%.

The characteristics of various IV IgG ion-exchange preparations were presented by the Canadians (Th-136). Data were given for specific and normal preparations:-

Preparation	IgG (g%)	IgA (g/g IgG)	PKA	Plasminogen	Anti-C	Agg. %	Fragments %
RH IG	1.0	32	0.1	<1	1.2	<1	<1
R IG	6.8	9	<0.05	6	4.3	<1	<1
VZ IG	3.0	10	<0.05	2	7.4	1.6	<1
T IG	8.3	8	<0.05	6	20.7	1.1	<1
ISG	2.0	11	<0.05	NT	4.6	1.2	<1
ISG	5.0	26	<0.05	<1	6.1	<1	<1

When questioned about the hepatitis risk, the author of the poster indicated that they were aware of the Ortho result (M-327) and were now planning their own chimpanzee studies. He also indicated that they still considered their specific IgG products safe (eg anti-D) as these involved only a small number of high quality donors.

5.2 Animal Studies of Side Effects

Bakker et al (Th-251) presented data from a rat model in which the hypotensive effects of various materials were studied. Rats were infused with 1ml/200g b.w./12 sec and the fall in blood pressure (ΔP) was recorded.

In a study of the effect of PKA the results were:-

Product	% PKA	% ΔP
Albumin	0	+4
Albumin	5	+3
I.M. IgG (NRC)	169	-40
I.V. IgG (NRC mark I)	0	-45
I.V. IgG (NRC mark I) + CI-INH	0	-50
I.V. IgG (NRC mark II)	0	-6

Heat aggregated IgG was specially prepared to test if ΔP was due to aggregates. The method of preparation was:-

IgG (original product)
|
Heat at 57°C (1st aggregate product)
|
Remove aggregates
(3.5% PEG pptn)
|
Dilution & ultrafiltration (PM10)
|
Heat at 57°C (2nd aggregate product)
& centrifuge

Results were:-

Sample	ACA (mg/CH50)	% ΔP	
Starting IgG	10	+2	(n = 14)
1st Incubation (8 hours)	0.04	-40	(n = 4)
PEG super	10	-5	(n = 4)
2nd Incubation (8 hours)	0.04	-50	(n = 3)
Final super	10	-5	(n = 4)

The effect of incubation time was also noted:-

Sample	57°C Incub. Time (hrs)	ACA (mg/CH50)	Aggregates (%)	ΔP (%)	
IV IgG (Mk I)	0	8.6	25	-45	(n = 7)
IV IgG (Mk II)	0	23.5	3	-8	(n = 21)
"	4	2.8	10	-30	(n = 3)
"	8	<0.04	15	-45	(n = 3)
"	12	<0.04	20	-40	(n = 3)
"	16	<0.04	25	-45	(n = 7)

Measurements were also taken for different concentrations of protein:-

Product	Protein Conc. Injected (%)	-ΔP at 4 mins	
NRC Mark I	3	38 ± 9	(n = 8)
	6	45 ± 14	(n = 11)
NRC Mark II, pilot	3	7.2 ± 6.3	(n = 9)
	6	9.2 ± 10.7	(n = 6)
NRC Mark II, routine	2 ± 2	3 ± 2	(n = 6)
Manufacturer B1	21 ± 20		
Manufacturer B2	6 ± 10	31 ± 20	(n = 7)

Conclusions from the study were:-

(i) I.M. ISG - induced hypotension
- generated bradykinin in vivo when PKA present
- severity of hypotension not related to BK generation in vivo

(ii) IV IgG - PKA non detectable but still sometimes long lasting hypotension.
- Correlated with concentration of aggregates.

(iii) PKA and IgG-aggregates may cause short and longlasting hypotension respectively.

(the following references were listed:- R. Rosevelt et al, J. Lab. Clin. med., in press and W. Bleeker et al, J. Lab. Clin. Med., in press).

In a hepatitis infectivity study of IgG prepared by ion-exchange (M-327) the test animals became infected (see2.4above). I was given the clear impression that the manufacturer of the ion-exchange materials (Pharmacia) and at least one other manufacturer of IgG (Rh Inst. Winnipeg) were aware of this result. Yet ion-exchange fractionation continued to be very strongly promoted throughout the congress and in his plenary lecture Curling (PL-1-7) proposed that chromatographic fractionation was superior to cold-ethanol fractionation for small scale (up to 500 l/wk) regionalised fractionation plants, particularly in developing countries (ie countries likely to have a high incidence of hepatitis in their population)!

5.3 Clinical Uses of I.V. IgG

5.3.1 Humoral Immunodeficiency

Cunningham-Rundles(S-19-3) gave the latest results from a study of Sandoglobin begun 2½ years ago. The study involved 1 year on I.M. IgG followed by 1 year on I.V. IgG and the patients response was assessed from both clinical illness and from daily personal diary records.

There are 43 patients in the study (23M, 19F, age 5-63 years), 32 patients had primary immunodeficiency and 65% had T-cell defects.

So far 21 patients have completed (i.e. 1 year I.M. and 1 year I.V.) and 10 patients are due to finish in 1982. 3 patients have been withdrawn because of reactions.

With the IV treatment (300 mg/kg/3 weeks at 1-3 ml/min) a mean plasma IgG level of 500-600 mg/dl was achieved by all but 5 patients. These 5 all had an initial plasma level below 50mg/dl and it was postulated that they required a higher or more frequent dose. (Note: on initial I.V. dose of 150 mg/kg was given to assess adverse reactors).

The formation of immune complexes was observed after 3 infusions, CIq increased to normal levels after 4 infusions while C3 stayed above normal. The immune complexes began to disappear after about 5 infusions and were only seen sporadically afterwards.

The clinical outcome of I.M. versus I.V. was:-

	Condition	Number of Episodes IM (1 year)	IV (1 year)
(i)	from diaries		
	Sickness	1131	327
	Fever	940	145
	Need for antibiotics	2980	1064
	Hospitalisation	61	41

	Condition	Number of Episodes IM (1 year)	IV (1 year)
(ii)	Clinical illness		
	Upper resp. infect.	1020 (18 patients)	622 (18 patients)
	Total infections	2473	1675

A comparison was also made between the first 6 months and 2nd 6 months of IV treatment.

Condition	Number of Episodes 1st 6 months	2nd 6 months
Fever	92 (8 Patients)	2 (2 patients)
Antibiotics	435 (10 ")	289 (4 patients)
Hospitalisation	41 (3 Patients)	0
Total Infections	837	520

From a total of 638 IV infusions there have been 18 adverse reactions (ie 2.5%). These were considered as:-

Reaction	No. Patients	No. Reactions	Comment
Mild	2	4	Infusion rate too high.
Moderate	2	4	Chills, fever etc responded to treatment.
Severe	3	8	Ix anti-IgA; 2 x IgM paraproteins.

It was argued that the 3 severe reactors should have been excluded from the study on the basis of their plasma profiles.

The interim conclusions from this study are that 70% of the patients have shown a distinct improvement and that this can be assessed after 6 months. However the improvement was progressive and was even more marked during the 2nd 6 months.

5.3.2 Septicemia in Premature Babies

Von Muralt (S-19-4) reported the use of IV IgG (Sandoglobin) in 35 cases of neonatal septicemia.

The dosage was Ig daily for 6 days, infused over 3 hours in 30ml saline. After 6 days,serum IgG levels were normal.

Comparison was made with untreated controls. For a wide range of clinical indicators IV IgG therapy showed only a marginal (if any) improvement. However a significant improvement was found for mortality (ie 11%/44%, $p = 0.04$) and for the incidence of recurrent infections (11%/27%).

There were no adverse reactions and no depression of humoral immunity.

It was then postulated that IV IgG should really be given to the mother before delivery. A dose of 3g every 6 hrs prior to delivery (ie 12g in 24 hrs) resulted in no improvement in the baby. Further studies are in progress using a higher dose (ie 0.5g/kg) in the mother.

5.3.3 Immunothrombocytopenia (ITP)

A number of reports were given (S-19-5, S-19-6, S-19-F, F-59) of IV IgG in ITP following the work of Imbach (Lancet i, 1228-31, 1981).

Imbach (S-19-5), giving a dose of 0.4 g/kg per day for 5 consecutive days (Sandoglobin) reported that all patients responded promptly but that progress was difficult to evaluate. In a randomised trial of IV IgG verses steroids 3 of 15 in the steroid group were still thrombocytopenic and the IV group seemed best.

Abe (S-19-6) reported results using 0.4 g/kg (Sandoglobin) per day for 5-7 days. Immune functions were normalised and there was a marked improvement in 6 of 9 patients. Equivalent results had been achieved with other IV IgG products but the study has been restricted by the expense of the products.

Bussel (S-19-7) gave results from 20 patients (15 adults, 5 children) receiving Sandoglobin (400 mg/kg/day for each of 5 days). The following side effects were noted:-

Mild headache	5 patients
Allergic Reaction	1 patient
Hypertension	2 patients

Increase in platelets was evident with increasing serum IgG (correlation coef 0.8). Increases were from 28 x 10^3 to 219 x 10^3 at days 5-8. 7 patients had a good response but 6 patients had a poor response, though even the poor responders achieved >20 x 10^3 platelets (considered the minimum satisfactory level).

Non-splenectomised patients responded early (ie <4 days) while splenectomised patients responded late (5-8 days). Increases in plasma IgM were seen on day 8. Clinical summary was:-

(i) All patients showed increased platelets.

(ii) 5 patients no longer needed therapy.

(iii) 7 patients required continuing booster doses.

(iv) 5 patients have ceased to respond to boosters.

(v) There was no toxicity or serious adverse reactions and IV IgG was therefore recommended for all patients.

Possible mechanisms of action postulated included:-

(i) Fc receptor blockage.

(ii) Decrease in anti-platelet antibody.

(iii) Protection of megakaryocytes.

In discussing these presentions the possibility of treating other autoimmune diseases was raised. Hassig said that he was treating an allergy but it was too early to give results while Cunningham-Rundles said that she had seen no response in a number of patients with autoimmune diseases but had achieved good results in 3 patients with neutropenia (see letter in N. E. J. Med).

5.3.4 Other Clinical Reports

Kornhuber (M-204) reported the prophylactic use of IV zoster IgG in leukemic children. The product characteristics were:-

Protein	15%
IgG (7S)	>95%
Varicella titre	>1:2000 CI
Anti-C	low

Administration by IV and IM routes were compared. IM dosage was 15% protein while IV dosage was 5% (Zig + 0.9% NaCl) at 2ml/min. There were a total of 314 IV applications and 21 IM and the interval between contact with varicella and passive immunity was <72 hours. Clinical results were:-

Incidence of varicella	4/335
Mitigated varicella	3/4
Varicella pneumonia	1/4
Severe side effects	0
Fatalities	0

The use anti-meningococcal IgG was presented by Pokrovsky (M-324). The dose was 0.5ml/kg given IM and this was said to have "eased the course of the illness".

5.4 Other Immunoglobulin Studies

Shibata (M-317) studied the half-life of anti-hepatitis Bs immunoglobulin prepared by different methods from the same plasma pool. IM-IgG produced a 30% increase at the 4th day and this declined with a half-life of 24 days. PEG IV-IgG (7S) produced 100% activity at 1 hour, fell to 30% at the 8th day and then declined with a half-life of 18 hours. Pepsin IV-IgG (5S) gave 50% activity after 1 hour, 10% at the 3rd day and a net half-life of 2.7 days.

IgG purification by means of a simplified cold-ethanol scheme was presented by Boros (Th-132) Fractions I, II, III and IV were removed together (pH 5.65, 40% ethanol, 4°C) and then reworked. Two rework methods were tested.

Method 1

FI, II, III, IV

pH 5.05, 1% protein, 14% EtoH, ionic str. 0.02, -3°C

Centrifuge ——— solids

pH 7.1, 25% EtoH, ionic str. 0.05, -7°C

ppt IgG

Method 2

FI, II, III, IV

pH 7.0, 1% protein, 19% EtoH, ionic str. 0.001, -6°C

ppt

pH 5.05, 1% protein, 14% EtoH, ionic str. 0.02, -3°C

Centrifuge ——— solids

pH 7.1, 25% EtoH, ionic str. 0.05, -7°C

ppt IgG

Product characteristics were:-

Parameter	From FI, II, III, IV		From FII + III
	Method 1	Method 2	
Purity %	89	97	98
Yield g/l	5.1	4.6	4.8
Polymer %	3.0	3.7	2.1
Stability %	5.5	2.2	4.1

Stephan presented a method for the preparation of intravenous IgM (TH-186).

Cohn FIII
|
| 3% Aerosil, 20°C, 4 hours.
|
Centrifuge ——— solids
|
| DEAE-Seph A-50
|
Supernatant
|
| $Ca_3(PO4)_2$ + octanoic acid
|
Supernatant
|
| Diafilter & concentrate to 5% protein
|
Supernatant
|
| Eliminate anti-C by modifying with 0.1% β-PL
|
Hydrolysis, diafiltration, ultrafiltration, concentration, Sterile filtration, filling.
|
IgG Concentrate
(IgG + IgA + IgM, 5% for intravenous use)

The product characteristics were:-

LOT	IgG	IgA (mg/100ml)	IgM	Anti-C (µg C (1:30) per mg protein)
OP 07081	3700	700	828	6-11
OP 03081	3850	755	610	6-11
OP 01081	3950	770	590	6-11

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6. ANTITHROMBIN III

6.1 Clinical Reports

Antithrombin III deficiency was discussed for both congenital (S-9.3, S-9-4, F-190) and acquired (S-9-1, S-9-5) deficiencies. The role of replacement therapy in congenital deficiencies seems to be accepted and is likely to be an area of growing demand. There was some disagreement about the incidence of this condition, with a figure of $100/10^6$ population being given for Hungary (S-19-4) but only $10/10^6$ population in the USA. Machin (Middlesex Hosp) later estimated the UK incidence to be $2/10^6$ population (excluding Aberdeen!).

Acquired AT-111 deficiency is now being noted in a number of clinical situations (Schipper et al. Thromb. Res. 21, 73-80, 1981) and Ten cate (S-9-5) gave an up-to-date report of this work. In the initial study 23.5% of surgical patients had been found to have an AT-111 level below 80% of normal. Of 50 patients in intensive care 39 showed AT-III deficiency and 29 of these died. The next stage of the study considered 174 post-operative patients. All patients showed a post-operative dip in AT-III. This recovered in those patients who did not develop septicaemia but not in those who developed gram -ve septicaemia. It was proposed that AT-III levels could provide diagnostic information to identify patients at risk and that replacement therapy with AT-III concentrate may be useful. Particular clinical situations proposed for AT-III therapy also included caesarean deliveries and premature infants. Similar observations concerning plasma AT-III levels were presented (S-24-5) in the Symposium on Fibronectin (see section 7).

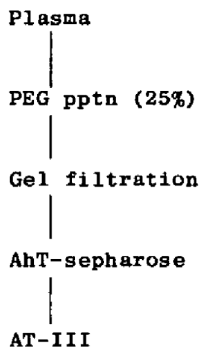
A question was posed by Machin concerning the quality of commercially available products but it was generally agreed that there was as yet insufficient clinical data on which to judge the merits of different products. It later transpired that Machin's concern was expressed in his poster (F-190) where it was reported that the concentrate used (Kabi) contained more antigenic than functional activity (eg heparin-binding).

This observation is almost certainly related to the method of manufacture. AT-III prepared from Cohn fractions is known to be highly complexed (Middleton, unpublished results 1975) and therefore likely to be inactive clinically. Concentrates prepared directly from the main plasma stream are therefore likely to be of a higher quality.

6.2 Preparation of Concentrates

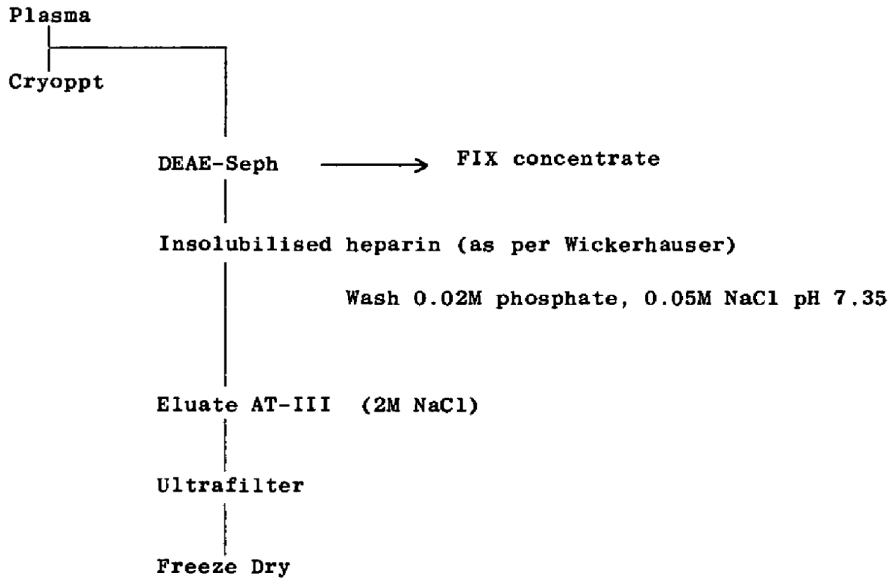
Two posters presented studies concerning the preparation of AT-III concentrates.

Tomono (F-181) reported a new anhydrothrombin (AhT) - sepharose affinity procedure. The preparative scheme was:-



The product had a specific activity of 3.8 and a purification of 190x plasma.

Mazurier (F-187) reported data from 24 batches of AT-III concentrate prepared to study batch to batch variations in antigenic and biological activity, to detect possible instances of denaturation and to test IN VIVO characteristics. The preparative method was:-



Albumin was added to 4 batches prior to freeze drying but appeared to have no effect. None of the batches were heat treated to inactivate hepatitis virus(es).

The mean results from 24 batches were:-

Anti-Thrombin III

by heparin-cofactor (chromagenic) activity (u/ml)	34.6
by progressive AT-III (u/ml)	42.2
by immunological activity (g/l)	11.4
% heparin binding ATIII-Ag	87.9
Specific activity (u/mg)	3.6
% α2 - migrating components	78.0

The conclusions were:-

(i) There was a good correlation between radial immunodiffusion and biological activity (ie heparin-cofactor & progressive activity).

(ii) The degree of denaturation was small.

(iii) Non-binding antigen had more thrombin neutralising activity than heparin-cofactor activity. Virtually all AT-III antigen exhibits progressive anti-thrombin activity.

(iv) In vivo recovery was satisfactory with a half-life of 96 hours for heparin-cofactor AT-III and 70 hours AT-III antigen.

(v) After infusion in congenital deficiencies the small amount of non-heparin-binding material disappeared.

7. FIBRONECTIN

7.1 Clinical Studies

There were a number of reports pursuing the theme of low plasma fibronectin levels in a variety of disease states. Brodin (M-137) reported a continuous fall in plasma fibronectin (to about 50% of normal) during, the first 3 weeks of chemotherapy for the treatment of acute myeloid leukaemia. A significant correlation was found between low fibronectin levels and signs of infection. A controlled trial of prophylactic administration of cryoprecipitate is underway.

Eriksen (S-24-4) gave details of plasma fibronectin levels in serious illnesses. In post-operative patients the fall in plasma fibronectin was in the order:-

(i) Sepsis (fall of 200mg/l, n=20)
(ii) DIC (fall of 200mg/l, n=10)
(iii) Other complications (-150mg/l, n=49)
(iv) No major complications (-100mg/l, n=30)

For the sepsis/DIC group there was no significant difference in plasma fibronectin before Sepsis/DIC, the lower levels developed during Sepsis/DIC and non-survivors showed a significantly greater depletion than survivors. The levels of fibronectin were then seen to increase during recovery. In burns patients a fall in plasma fibronectin was noted when complications developed (eg Sepsis) while in surgical patients the level of fibronectin was related to the extent of the excision or the amount of blood transfusion. Significantly low levels of fibronectin were also noted in chronic renal failure but this increased during dialysis. Erikson concluded that, except for the Sepsis/DIC group, it was difficult to relate the clinical condition to plasma fibronectin.

This point was taken further by Rubli (S-24-5) who introduced her report by saying that their conclusions were now somewhat different to those given in the abstract. Rubli et al have studied the behaviour of 6 proteins in 127 patients. All patients were in surgical intensive care, 97 of whom were non-septic and 30 with severe infections. The proteins screened were:-

Fibronectin
Anti-thrombin III
IgG
Transferrin
C3
Pre-albumin

For the septic patients there was a significant fall in fibronectin, antithrombin III, transferrin and pre-albumin with no difference for IgG and C3. In comparing septic survivors versus non-survivors (mortality 42.4%) there was no correlation with fibronectin levels but there was a very strong correlation with AT-III levels (ie 67% mortality for AT-III <50%). However, if fibronectin levels were very low then mortality was high (ie 80% if < 200 mg/ml and 31% if > 200mg/ml) nevertheless a number of patients suffered fatal infections even though their fibronectin levels were normal. In summary, low fibronectin levels were confirmed in septic patients but AT-III, transferin and pre-albumin were also low. Minimum levels of fibronectin were not predictive for survivors. Mortality was high if AT-III was < 50% and if the maximum fibronectin level was < 200 mg/ml. The conclusions from the study were:-

(i) Fibronectin levels were not predictive.

(ii) The acute depletion of fibronectin was not an isolated phenomenon but only part of a process of acute plasma protein depletion.

(iii) Although trials involving fibronectin are underway the situation is more complex than previously thought and the trials must be very carefully controlled.

(iv) Conquering the underlying infection may be the best approach, rather than replacement therapy.

In discussion some participants felt that these new complexities did not match up with the reports of successful cryoprecipitate therapy. It was accepted however that controlled trials of cryoprecipitate replacement had yet to be undertaken, as had controlled trials with fibronectin, AT-III, transferrin etc.

There was some speculation on the functional role of fibronectin. Clemmensen (S-24-2) stressed tissue repair, opsonic activity and antithrombin activity while Lundsgaard-Hansen (S-24-3) remarked on "good results" from infusion of fibronectin in animals and postulated that fibronectin may have a number of different functional activities all of wich required specific assays. These functional assays were needed for patient studies and to control the preparation, storage, etc of concentrates, as some of the biological functions may be very sensitive. References listed were:-

Scorville, Ann. Surg. 188, 521, 1978
Scorville, Surgery 86, 584, 1979
Arnest, J. Trauma 20, 726, 1980
Robbins, Am. Surgeon 46, 663, 1980

7.2 Binding and Elution

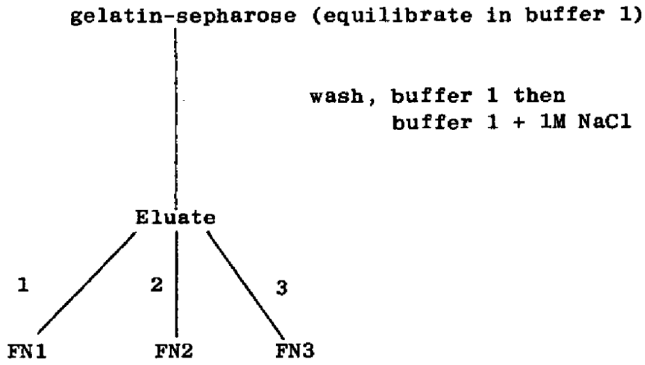
Cseh (M-328) studied the binding of purified fibronectin (purified by gelatin-seph, arginine-seph) to various potential components of cryoimmuno-globulins and immunocomplexes. Significant binding was found for (in order):- gelatin > IgG3, > CIq, CI, IC/CIq, > IgG1. IgG2 and IgG4 were not bound to any extent.

Different chromatographic procedures for the preparation of fibronectin were studied by Henon (Th-252). Four systems were compared, gelatin-sepharose adsorption with 3 different elution buffers and heparin-sepharose adsorption.

The preparative details were:-

Buffers: buffer 1 - tris Na citrate 20mM, pH 7.4
buffer 2 - Na citrate 50mM, pH 5.0

Methods



Conditions

1. Gelatin-sepharose, elute with buffer 1 + 8M urea
2. Gelatin-sepharose, elute with buffer 1 + 1M Arginine
3. Gelatin-sepharose, elute with buffer 2
4. Heparin-sepharose.

Results

Method	Yield	% Purity		
	%	by Rocket	by Laser nephelometry	by ELISA
FN1	45	87	80	77
FN2	40	40	25	32
FN3	15	85	71	79
FN4	not given	81	81	74

Contaminants were identified using specific antisera:-

Method	Contaminated With			
	Fibrinogen	IgG	Albumin	FVIII
FN1	+	0	+	0
FN2	++	+	+++	0
FN3	++	0	0	0
FN4	++	0	0	0

It was noted that product FN4 had lost part of its ability to bind collagen and that method FN1 gave the best results in terms of yield and quality.

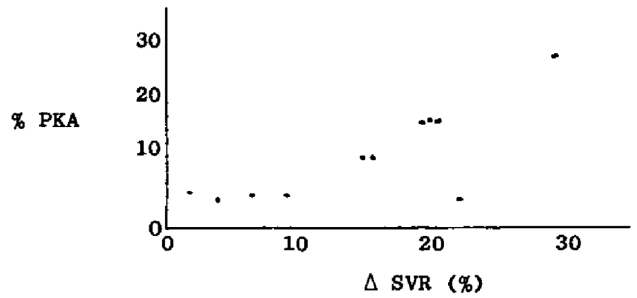
In private discussion concerning problems of leaching from affinity systems Walmsley (Pharmacia) indicated that a small degree of leaching was experienced with the use of even fresh reagents and that this comprised a complete soluble portion of the ligand-sepharose complex. This was said to be barely detectable, but detectable breakdown/leaching was likely after re-using the reagent a number of times. The point of breakdown was likely to be different for different requirements. On the same topic, Harvey (BPL) indicated that a more firmly bound affinity reagent for fibronectin was under development at BPL, but that details could not be given because of the patent situation.

8. ALBUMIN

8.1 Adverse Reactions

Peltola et al (Th-124) reported a clinical study of open-heart surgery using a variety of albumin preparations. The test solutions included albumin prepared by different methods (eg ion-exchange, cold-ethanol) and with different levels of PKA, sodium, glucose and osmolality. The solutions produced no significant decrease in mean arterial pressure but decreases in the systemic vascular resistance (SVR) were observed (Heinonen et al Ann. Thor. Surgery, 33, 244-249, 1982).

A correlation was observed between PKA content and the fall in SVR (see sketch figure)



Only 1 point fell outwith the relationship (4% PKA, 21.9% ΔSVR) and this solution had the lowest osmolality (210 mOsm/kg). Adjustment of the osmolality with either NaCl or glucose did not effect the haemodynamic response and NaCl was preferred because of the potential for glycolysation of albumin.

It was concluded that PPF-solutions with low PKA and physiological osmolality and sodium content were safer plasma expanders than the other tested solutions. No differences were observed between the different methods of manufacture.

8.2 Preparative Methods

Boros (Th-132) reported the preparation of albumin by a 2-stage cold-ethanol procedure similar to that of Hao. i.e.

Stage 1
pH 5.65
5% protein
40% EtoH
-4°C
Stir for 6-10 hours
Centrifuge

Stage 2
Super
Filtration (EK, 0.1% hyflo super cell)
pH 4.8
40% EtoH
-10°C
Stir 6-10 hours to ppt albumin

Purity was given as 98.2% and yield as 24.4% (sic) - probably 24.4 g/l plasma. Both Eriksson (Th-125) and Harvey (Th-129) reported the recovery of albumin from FIV. Eriksson et al indicated a 10-15% loss into FIV and they were able to recover 100g albumin/kg paste by ion-exchange chromatography using 5kg batches FIV. PKA values were relatively high (19.5% for batches 1 & 2, 12% for batches 3 & 4) even though the albumin purity was good (99.5%) (details of the method are available in a reprint of the poster).

Harvey presented his affinity chromatography method using Cibacron blue-sepharose. With 14.3mg dye/g sepharose, a capacity of 40mg albumin/g adsorbent was achieved. This has been scaled-up to a 1000 cm^2 column (bed height 16 cm) with a capacity of 300g albumin. Fraction IV was said to contain 75g albumin/kg FIV and the chromatographic process was therefore able to handle 130kg FIV per week. Toxicity studies are almost complete and these will be followed by clinical trials.

Eriksson et al (Th-126) presented another chromatographic procedure for the removal of haemoglobin from albumin solutions. The colour index (E403) of the product was reduced from 0.245 to 0.09 and the ion-exchange yield was 98% (a reprint of the poster is available).

There were a number of presentations concerning albumin preparation by chromatographic (Pharmacia) processes. The procedure of Berglof (Th-128, reprint available) involved 1 precipitation, 4 chromatographic and 2 ultrafiltration stages for albumin alone or 2 adsorption/centrifugation, 5 chromatographic and 2 ultrafiltration stages for albumin, if IgG is also required. Plasma pools of up to 60 litres have been handled this way and the yield was 26.5g albumin/l plasma (n = 9). For desalting and concentration the combination of gel filtration and ultrafiltration was said to be preferable to diafiltration and ultrafiltration because of

the length of time required for diafiltration.

Other reports were from France (Th-127), Yugoslavia (Th-130), South Africa (Th-131) and Canada (Th-136). There was a suggestion that one country was experiencing a pyrogen problem, but another country (Th-130) reported a rabbit response in the pyrogen test of 0.00°C.

The automation of chromatographic systems was presented (Th-139). Parameters controlled were UV (280 nm), pH and ionic strength. The most interesting feature was the in-line measurement of ionic strength "with an original system without any effluent contact with any electrode ... this avoids all electrolytic phenomena". Details of this aspect were not provided but from the photograph of the unit a flow-cell apparatus seemed to be involved.

9. PLASMINOGEN

Abe (F-152) reported the use of plasminogen for thrombolytic therapy of cerebral infarction. Human placental partially activated plasminogen (lys-Plg, Substrene, Lab. Choay) was used. Lys-Plg was infused alone and in combination with urokinase (UK) in studies with healthy volunteers and patients.

For phase I (health volunteers) four regimes were studied:-

(i) Single dose Plg:- 15 x 30& 60mg Lys-Plg to 3 volunteers.

(ii) Multiple dose Plg:- 15 x 30 & 60mg Lys-Plg daily for 4 days.

(iii) Single dose Plg + UK:- 30mg Lys-Plg then 30000 iu UK.

(iv) Multiple dose Plg + UK:- 30 & 60mg Lys-Plg then 30000 iu UK daily for 4 days.

Conclusions from phase I were that fibrinolysis was enhanced without any evidence of side effects.

In the clinical study (phase II) 48 patients with cerebral infarction have been treated with Plg + UK or Plg alone. The regimes were:-

(i) 60mg Lys-Plg in 5% glucose (250ml) infused over 30 minutes. After 1 hour 30000 iu UK were infused in 5% glucose (250ml) over 1 hour. This was repeated daily for 4 days. or

(ii) 60mg Lys-Plg in 5% glucose was administered daily for 7 days.

It was concluded that, for both groups, patients with slight or moderate severity or with a shorter elapsed time (< 15 days) showed highly effective clinical results. The rate of improvement was more remarkable on day 7 than day 4 and there were no serious side effects except for 1 case of gastro-intestinal haemorrhage from the Plg + UK group.

10. ENDOTOXIN DETERMINATION IN BLOOD PRODUCTS

Visser (Th-249) described the use of a chromogenic substrate (S2423) in the limulus assay for endotoxin. For protein solutions the following pretreatment was used:-

(i) Dilute 1/10 with buffer to a final concentration of 40mM tris/HCl, pH 7.45, 0.8mM $CaCl_2$ in disposable polypropylene tubes with air vent.

(ii) Incubate for 10 minutes at 100°C (80°C) in a heating block.

(iii) Centrifuge (2000g, 5 mins, 20°C) and use supernatant for test solution.

Procedure

	Manual	Automated
Volume sample, blank, standard (µl)	50	100
Volume LAL reagent (µl)	70	175
Incubation time (37°) to activate LAL (min)	45	30
Volume chromogenic reagent (µl)	500	250
ΔOD at 405nm	Photometer	Discrete analyser

In studying the extraction of endotoxin from protein solutions the following points were noted:-

(i) Endotoxin was completely extracted by heat denaturation of the protein.

(ii) Heat coagulation was critically dependent on the calcium concentration of the extraction medium.

(iii) Calcium was also required to neutralise citrate contamination of plasma protein derivatives.

(iv) Excess calcium is inhibitory for activation of the amidolytic enzyme activity.

(v) 10 mg% pyrospers (Mallinckrodt) in the extraction medium during heat denaturation promotes the extraction of endotoxin. This is essential for the extraction of endotoxin from FVIII concentrate.

11. CELLS

11.1 Cell Growth

There were a number of posters concerning growth factors etc (Tu-28 to Tu-46) Ogasawara (Tu-30 and Tu-31) described the identification and isolation of a colony-stimulating-Factor-Helper-Factor (CSF-HF) obtained from conditioned medium of bone marrow cells cultured with endotoxin. After examining various strategies for isolation CSF-HF was found to adsorb to DE-53 (pH 7.5) and could be eluted (between BSA and Cyt. C) using a 0.5M NaCl gradient.

Colony stimulating activities (CSA) were also isolated from human placental conditioned medium (HPCM) by Wolkersdorfer (Tu-35). The method of preparation of HPCM was given as:-

(i) The placenta was collected by caesarean section and placed immediately in Hanks balanced salt solution containing penicillin (100000 UE), streptomycin (100mg) and amphoterician (25mg) (PSA)/1.

(ii) The tissue was cut into pieces (5g), rinsed with NaCl (0.15M), then finely chopped and cultivated at 37°C in a fully humidified atmosphere with 5% CO_2 in RPMI 1640 containing PSA/1.

(iii) The cellular elements were removed by centrifugation after 7 days. The supernatant was sterile filtered and stored frozen at -196°C. This material was basic HPCM which was further treated.

(iv) HPCM was dialysed against large volumes of sodium phosphate (0.01M, pH 6.8, 4°C).

(v) Dialysed HPCM was adsorbed with calcium phosphate (2 hr at 25°C). The supernatant was collected and the solids washed with sodium phosphate (0.05M, pH 6.8, 25°C).

(vi) The supernatant and wash solutions were pooled and freeze dried. Resolution was in PFW at 1/5 original volume.

On gel filtration (Seph G-75) two different activities (CSA) were found CSA1 28000 MW and CSA2 13500 MW.

11.2 Cell Products

Monoclonal antibodies were reviewed in plenary session PL2. Koskimies (PL-2-5) noted that antibody production from EBV transformed lymphocytes stops after about 7 days for selected cells or after 3 days for non-selected cells. Cells for anti-D were said to be producing IgG 1 only. In his review of interferon Friedman (PL-2-8) said that 16 kinds of α-interferon had been identified, that β-interferon was stable at pH2 and that γ-interferon was unstable. Clinical studies on the effect of interferon on tumours are due to be reported in 6 months time and new disease states are begining to attract attention (eg Rheumatoid Arthritis). Nevanlinna (PL-2-9) was unhappy that the Finnish Red Cross was now excluded from information in this area and that there seemed to be little interest in leucocyte interferon.

In his review of hepatitis B viruses Vyas (PL-3-3) indicated that monoclonal antibodies to hepatitis B showed some fluorescence with non-A, non-B material and he postulated that this antibody may posses more specific sites for the surface antigen than human antibody.

A monoclonal antibody to FVIIIR-Ag was described by Goodall (F-88). The method for preparing the immunogen was:-

Factor VIII Concentrate in H_2O

|

Sepharose 6B, 0.1M Citrate-saline, pH 7.3

|

Void vol. fractions pooled and concentrate with 12% PEG 6000

|

ppt reconstituted in barbital-saline, pH 7.4.

12. COAGULATION FACTORS (MISCELLANEOUS)

12.1 Factor VII

Karpati (F-97) studied the activation of FVII by granulocyte protease, concluding that:-

(i) Small amounts of protease (ie 0.25 Cas mU/ml) induced rapid transient activation of FVII. This quantity of protease is equivalent to that of 7.4×10^4 leukocytes.

(ii) Calcium (2.5 mM) protects FVII from inactivation and results in a higher degree of activation.

(iii) Granulocyte chymotrypsin was more effective than granculocyte elastase.

In discussing the poster the author said that the system required granulocyte chymotrypsin specifically and that chmotrypsin from other sources was not effective.

Abildgaard (F-100) reported that FVIIa is inhibited by normal plasma. Further investigation showed that about 1/3 rd of the inhibition could be accounted for by AT-III. The remaining inhibitory activity was directed against both FVII and FVIIa. Gel filtration of normal plasma indicated that there were 3 different inhibitors other than AT-III.

12.2 Protein C

Hashimoto (F-103) studied the behaviour of protein C in plasma and noted that:-

(i) Protein C is activated by thrombin.

(ii) The addition of activated protein C to plasma gave an increase in the APTT.

(iii) Protein C activation by thrombin and $CaCl_2$ in the presence of umbilical cord homogenate corresponded with a selective inactivation of FV and FVIII.

(iv) In DIC patients the fall in protein C decreased according to increasing FDP.

(v) Plasma protein C levels decreased during Warfarin treatment.

(vi) Plasma possesses a protein C inhibitory activity (PCi), but there was no correlation between PC & PCl levels in DIC.

It was concluded that protein C has a genuine In Vivo function in coagulation and that this may be important in patients with hypercoagulation.

Seghatchian (S-23-3) indicated that protein C activation required thrombin and a cofactor from endothelial cells.

12.3 Other Points

Varadi et al (F-101) considered the effect of proteolytic enzymes and calcium on FIX and FX. They concluded:-

(i) FIX and FX are about 6-10 fold more resistant to proteolysis (by granulocyte proteases) than when in their activated state.

(ii) The rate of inactivation of FIX and FX was decreased in the presence of calcium (2.5 mM).

Calcium also protected FIXa and FXa against proteolysis for up to 15 minutes.

(iii) The protective effect of calcium was attributed to a conformational change in the protein structure.

In discussion future problems in the treatment of haemophilia, Aledort (S-23-1) reported that the most recent problem to surface in the USA has been 3 deaths from pulmonary infections. This has been linked with the development of Acquired Immunodeficiency Syndrome.

13. OTHER TOPICS

The preparation of a transferrin concentrate was reported by Jakab (Th-133). The method involved adsorption with DEAE-Seph A-50 (0.05M tris/HCl + 0.05M NaCl, pH 8.6). Transferrin was eluted with 0.05M tris/HCl + 0.075 NaCl, pH 6.1. The final product was 97% pure with a protein concentration of 1.6g/l and a yield of 43%.

Burnouf et al (Th-138) described a continuous chromatographic procedure for plasma fractionation based on the Trisacryl matrix.