IN CONFIDENCE

MINUTES OF MEETING OF FACTOR VIII STUDY GROUP HELD IN HEADQUARTERS UNIT, ELLEN'S GLEN ROAD, EDINBURGH ON THURSDAY, 28TH JANUARY, 1982

Present:

Dr J D Cash (Chairman)
Dr F E Boulton, Edinburgh Centre
Dr C V Prowse, Edinburgh Centre (Secretary)
Dr D S Pepper, HQ Laboratory
Mrs B Griffin, HQ Laboratory
Mr J G Watt, Protein Fractionation Centre
Dr P Foster, Protein Fractionation Centre
Dr R J Perry, Protein Fractionation Centre
Dr G S Gabra, Glasgow (Law) Centre
Mr A Farrugia (in attendance)
Mrs E Porterfield (notes)

1. Dr Cash opened the meeting with a brief resume of the need for convening such a group, setting out the aims which might be achieved. It was agreed that one representative from each Centre would speak on the work currently underway in his/her Centre and research/development proposals for the future.

2. EDINBURGH CENTRE: DR C V PROWSE

The current activities in Edinburgh BTS were outlined in some detail with the use of slides. The desired end product was more and better factor VIII, there being several ways this could be achieved. These were summarised as follows:-

(A) More from Donors

(i) More donors
(ii) Plasmapheresis
(iii) More plasma factor VIII per donation - more red cell concentrate
- use SAG red cells etc.
- treat donors with DDAVP.

(B) Improve Processing

(iv) Plasma collection - collection in heparin
- faster freezing
- special packs

(v) Cryoprecipitate - thaw siphon/continuous thaw
- heparin enhancement
- freeze drying as final product

(vi) Further purification - cold precipitate ± heparin
- column chromatography
- prevent degradation

(C) Other Sources

(vii) Animal plasma

(viii) Bio-engineering: (a) VIII RAg
(b) VIII:C - ?source specific RNA/DNA

(D) Safer Products

(ix) Viral inactivation - pasteurisation
- irradiation
- BPL -UV

(x) High purity.

Edinburgh had carried out studies on thaw siphon cryoprecipitate which had produced a yield of 700 u/l but some purity/potency had been sacrificed in the frozen product. Tests had been carried out on triple donor pools which showed product was clinically acceptable, yield from Group A being 800 u/l and from Group O 600 u/l. There is some doubt as to whether this observation of blood group differences should be pursued as the same yields are not apparent in small scale (2 ml) quick thawing.

Inverted thawing had been tried and a purer, higher potency product is obtained with this method but, yield is reduced to 500 u/l.

Two studies had been carried out to assess survival and recovery in haemophiliacs. Study A compared overnight thaw cryo; thaw-siphon cryo and PFC intermediate concentrate. Results showed that all three had a similar half life. Although recovery was higher for concentrate, initial removal was possibly faster. Study B (currently underway) compares Group A thaw-Siphon cryo with Group B thaw-siphon cryo and Glasgow freeze dried cryo. So far no gross differences are evident.

Studies to assess the FVIII content of PFC feed plasma using BTS and PFC core samples gave a best result of 840 u/l in CPD plasma frozen within 6 hours of donation. FVIII content had shown a recent drop for which there was no obvious reason. A graph showing production losses is attached at Appendix I. It is known that mechanical and degradative changes during cryoprecipitation cause initial losses. Thereafter, during adsorption, filtration and drying, further degradation occurs. However, losses were improved on continuous bulk thawing. Reference was made to the studies carried out by Dr Gail Rock on heparin addition which claimed an increase in plasma VIII level x 1.5 (heparin) and x 1.3 (heparin-CPD). The latter had been obtained by one laboratory only and had not been confirmed. The factor VIII yield in cryoprecipitate prepared from such plasmas were approximately 900 u/l (heparin) and 700-750 u/l (heparin-CPD) with possible improved stability. Further purification by cold precipitation in the presence of heparin can be carried out possibly due to the presence of fibronectin, in which case yield is slightly reduced.

Dr Prowse also outlined the work currently being carried out by Mr A. Farrugia in Edinburgh BTS, studying assay problems in the presence of heparin and looking at the question of timing of the addition of heparin. No definite results are yet available but it is possible that heparin may require to be added quite rapidly to the donor pack.

The work currently being carried out by Dr Prowse in collaboration with the HQ Laboratory on the binding capacity of FVIII to insolubilised phospholipids was then put to the group, who were already aware of the work by Anderson in this area. The Edinburgh work involves coating silica beads with phospholipids and examining the VIII binding. The results are shown at Appendix II.

3. PROTEIN FRACTIONATION CENTRE: DR P FOSTER

Dr Foster had split his talk into several sections and again made use of slides to illustrate many of the PFC studies.

The first section was given over to yield and Dr Foster briefly outlined the process used at PFC. In 1975 factor VIII content of plasma had been 660 iu/l. and after several years of liaison with other Centres, collaborating on problems etc., this had improved to 840 iu/l. although, as previously pointed out by Dr Prowse, this figure had fallen in 1981 to 700 iu/l. Cryoprecipitate yield had also improved from 320 iu/l to 500 iu/l which could be related to the development of the continuous thawing method.

FVIII concentrate yield had increased from 250 iu/l (1975) to 300 iu/l (1981) after processing and from 190 iu/l (1975) to 270 iu/l (1981) at issue. Specific activity was now 0.38 iu/mg. (had been 0.25 iu/mg. in 1979).

Again the question of losses during processing was looked at. It had been thought losses could be due to the disruption of VIII by $Al(OH)_3$ or entrapment of VIII on filters. However, antigen studies suggested that inactivation was taking place. Therefore, PFC had begun to look at possible reasons for such inactivation. Samples had been taken from process and FVIII:C levels evaluated after each stage of process over a large number of batches. At stage 1 (cryo extract) incubation for 2 hours revealed no change in activity from the initial value. Similarly, after $Al(OH)_3$ adsorption there was no significant loss after 2 hours incubation, but following the third stage (addition of citrate) loss of factor VIII occurred during incubation. After 1 hour the loss was approximately 7%; after 2 hours 14% and after 3 hours 18%. In the next stage the solution is filtered and is then left for 1 hour due to assay requirements before vialing. This process lasts for 2-3 hours and therefore citrate-induced losses of 15-20% are to be expected on the basis of the above experiments, and account for about 60% of the observed losses. Dr Foster pointed out that citrate is used by all manufacturers except Parke-Davis who use phosphate.

Studies on recent batches to which the normal 20 mM citrate was added were carried out and revealed a mean citrate induced loss of 15% prior to filtration. If citrate was not added a reduced loss of VIII was observed but the product was less soluble and tended to form clots. The full results are attached at Appendix III and suggest lower citrate levels may be optimal. It is intended to try other buffer/anticoagulant solutions for their effects on product stability etc. and the studies are already underway.

Dr Foster then showed the results of tests on crushed plasma for VIII content over an 18 month period. These had been carried out on pools of 6 hour, 6 and 18 hour combined and 18 hour plasmas. Progressive losses of FVIII:C were noted with delays in freezing. A graph had been drawn on a scale 0.25-1.50 u/ml on which VIII content fell as low as 0.4 from 1.09. Over the next few months this increased gradually to 0.8 u/ml. During the same period FVIII RAg results showed marked changes in both directions, peaking at 1.50 u/ml and falling as low as 0.3 u/ml. before "spiking" to 1.4 u/ml. Over the next few months the same pattern was repeated. Similar results had been found in Glasgow and Edinburgh. Insufficient data is available to explain these findings. Several possibilities exist - assays of starting plasma; seasonal variations; the assays need checking? Dr Pepper was of the opinion that RAg assay certainly required checking. Dr Prowse stated that part of the problem was probably due to the lack of organisation of the assays and that a more formal approach was required for long term studies of this kind.

The next area to be looked at was possible areas for improvement of FVIII yield. Studies on Haemonetics plasma from Leeds BTS collected in different anticoagulants had been done, to see if there were any beneficial effects of such changes. Freezing equipment was also investigated along with the crushing operation. There was some evidence that if crushing occurs at different temperatures yield and quality is dramatically affected. Particle size on entry to thawing vessel could also have some bearing, and this will be studied in the future.

More work is to be done on cryoprecipitate continuous thawing. Automation to be looked at and consideration to be given to increasing the scale for larger pool sizes along with the question of possible higher yields with heparin addition.

So far as adsorption stage losses are concerned, alternatives to $Al(OH)_3$ had been tried; although loss also occurred with these, it was reduced. There was/

was a suggestion that if starting plasma is very good and thawing also good VIII loss during adsorption was reduced. Careful specifications for starting plasma may therefore be very important. This area requires a vast amount of work on pyrogenicity, stability, adsorption, aggregation and sterile handling.

Areas to be investigated in improving the quality of the FVIII product have been identified as an improvement on purity/specific activity/low fibrinogen product together with improved solubility/dose volume. Removal of microbidogical/viral/pyrogenic contaminants also come under this heading.

The group was extremely interested in a graph showing specific activity of FVIII:C in relation to plasma age from a starting pool of Leeds BTS plasma (Appendix IV). The specific activity did not change much in relation to plasma age but fibrinogen content showed marked change. Each process stage requires to be examined carefully.

Dr Foster ended his talk with a resume of PFC R & D current project priorities which were:-

1. Reduce inactivation in process, e.g. alternative buffers
2. Develop high purity methods - e.g. metal ion pptn.
3. Reduce fill volume - ultrafiltration
4. Effect of plasma age - precipitate with reduced fibrinogen content
5. Anticoagulant effects on plasma FVIII; cryo FVIII.

(At this stage Mr S Keddie demonstrated the Pritchard airblast freezer. This machine costs about £12,500 and is capable of freezing single donor plasma to -40°C in 75 to 90 minutes at a rate of 60 donations/hour).

4. GLASGOW (LAW) CENTRE: DR GABRA

Dr Gabra began with an outline of the West commitment. Usage over the last 12 months had fallen to 8,000 packs of frozen cryoprecipitate, in line with a reduction over the last few years.

Opinion in the West was that dried cryoppt. (1) stored better, (2) was simpler to handle, (3) was safer, because (4) dose was specified on the bottle and (5) could be used by patients of any ABO group. Because of this the West planned to widen usage and offer this product to clinicians on a named patient basis as well as to selected patients on home therapy. At the moment the freeze dried material was mainly used for cardiac surgery, paediatric patients on home therapy and for Von Willebrand's patients. It was hoped it might be possible to offer the product on a named patient basis to the whole of the SNBTS. The product can now be produced from 5, 10 or 30 donation pools and is produced now as 400 u vials for resolution in 50 mls.

Freshness of plasma and early freezing would be looked at in an effort to improve procedures. Four experiments using plasma with red cells (i.e. whole blood) and separated plasma from the same donors stored for 4, 6 and 24 hours, testing for VIII:C, cryo VIII:C and VIII RAg showed no significant differences in decay rate or cryoprecipitation of VIII. Cryoppt. produced from heparinised plasma did not dissolve but with the addition of citrate reasonable results were obtained. Plasma collected in heparin, citrate and heparin/citrate combined showed no differences in factor VIII content.

Pooling procedures were outlined. Experiments had been carried out on one pool using different fluids, ranging from distilled water to glycine citrate as the resolution and pooling fluid. Measurements were taken of pH, VIII:C and VIII RAg. The results obtained, which were the mean of 3 runs, provoked a great deal of discussion. (Appendix V).

The fibrinogen content of the Law material had been assayed in 3 different laboratories (Law, PFC and HQ) and the results are shown below -

Assay	Mean content/bottle
Law	514.25 ± 110.18 mg.
PFC	742.5 ± 193.49 mg.
HQ	343.5 ± 63.42 mg.

(This represents 15-25% of the total protein content)

For the future Law's plans are not clear and cannot be defined until the future of the freeze drying plant is settled. For the moment plans are to continue to try to improve quality and widen clinical use.

5. HEADQUARTERS LABORATORY: MRS B GRIFFIN

Mrs Griffin had been asked to present the HQ Laboratory work in this area which she summarised as follows:-

1. Artifical haemophilic plasma (freeze dried) had been produced by mixing human serum, Kabi fibrinogen and bovine factor V. This was adequate for assay of plasma factor VIII but in the concentrates some discrepancies had been noted. Later work suggested that predilution in congenitally VIII-deficient plasma or EDTA treated human plasma avoided such discrepancies.

2. A large variety of adsorbents (e.g. collagen agarose) had been tested in an effort to remove fibrinogen from factor VIII preparation. Unfortunately, none of the adsorbents allowed adquate separation of factor VIII and fibrinogen.

3. Purified human VIII RAg had been prepared and injected into sheep at Law. The resultant antibody, after suitable adsorption, appeared monospecific and had been used to purify VIII:CAg by Ca elution of this from immobilised anti-VIIIRAg following adsorption of factor VIII preparation. Some of the antibody may be available from the HQ Laboratory on request.

4. Purified VIII:CAg from (3) had been immobilised on agarose as a solid phase adsorbent to allow screening of monoclonal antibodies to VIII:CAg. Initial results following immunisation of mice with porcine factor VIII were disappointing but further immunisation with porcine and human material were underway. Work on the purification of VIII:CAg was being pursued.

5. Recent experiments with controlled pore glass and bentonite (which adsorbs fibrinogen but not VIII:CAg but possibly activates VIII:C) were encouraging as methods for removing residual fibrinogen from VIII:CAg preparations.

6. An immuno-radiometric assay for VIII:CAg had been set up. This was an improved and simplified version of the one used at the Royal Free Hospital in London.

7. Phospholipid coated beads were being tested jointly with Edinburgh BTS as a reagent for factor VIII purification (see Appendix II).

8. Experiments on irradiation of dried factor VIII concentrate as a method of inactivating viral contaminants suggested that some loss of VIII activity occurred at about the same dose (2.5 Mrad) as is required for viral inactivation. Higher doses resulted in an insoluble product - this effect might be avoided by preliminary fibrinogen depletion of the concentrate prior to irradiation.

6. DR CASH

Dr Cash spoke on the proceedings, thanking all for their efforts, and suggested that in future the various Centres should be working more closely together. He asked for the views of those present on the necessity of setting up a group to study problems etc. With only minor qualifications/suggestions it was agreed by all that an SNBTS group was essential. Dr Cash then put his view of what required to be studied:-

(a) Assays (standards)
(b) RTC quality of plasma
(c) PFC
(d) Safety (e.g. irradiation as a means of viral inactivation)

No progress could be made until (a) had been solved. To this end the following was suggested:-

(i) Every Centre should be using the same assay. There should be a SOP for this assay.
(ii) Dr Cash would write to Transfusion Directors with proposals.
(iii) Small group to be set up to decide what was required. Dr Cash would be happy to join this group to liaise with the Transfusion Directors.
(iv) Dr Foster's slides were very relevant to this point and Dr Cash would attempt to find resources to enable work to be carried out.

There was general agreement that small groups required to be set up to study these areas and the following was decided:-

GROUP A

Assays (Standards) - Aim - To operate standard plasma assay in RTCs

Members: Dr C V Prowse (Co-ordinator)
Mrs B Griffin
Mr T McQuillan, PFC

GROUP B

RTC Quality of Plasma - Aim - To look at problems using assays devised by Group A; specification of plasma quality; recommendation of plasma freezing and storage conditions, etc.

Members: Dr G S Gabra (Co-ordinator)
Dr F E Boulton
Dr J D Cash
Mr S Keddie, PFC (freezing, storage, temp. control etc.)

GROUP C

PFC Product Development - Aim - To improve yield (at PFC) and purity of the PFC Product.

Members: Dr P Foster (Co-ordinator)
Dr D S Pepper
Dr C V Prowse

GROUP D

Safety - Aim - To improve the safety of the product, e.g. by irradiation to remove viral infectivity.

Dr D S Pepper (Co-ordinator)
Dr R Somerville
Mr B Cuthbertson

For the moment it was agreed alternative methods of factor VIII production, e.g. genetic engineering, would not be actively pursued but members should consider the effect and possible application of such techniques within the SNBTS.

7. DATE OF NEXT MEETING

It was agreed that the next meeting would take place on 31st March, 1982 commencing at 9.30 a.m. in the Headquarters Unit.