IN CONFIDENCE

Minutes of Factor VIII Study Group
held in the Headquarters Unit
on 7th February 1985

Present:
Dr J D Cash (in the chair)
Dr R J Perry
Dr P R Foster
Dr B Cuthbertson
Dr D S Pepper
Dr J Dawes
Dr F E Boulton
Dr C V Prowse
Dr G S Gabra
Mrs E Porterfield (notes)

1. INTRODUCTION

Dr Cash welcomed the members of the group and extended congratulations
to Dr Perry on his recent appointment to the post of Director, PFC.

He intimated that the order of discussion would be different to that
itemised in the agenda. For this reason the figures in brackets at
each heading will indicate the original item number.

2. MINUTES OF THE PREVIOUS MEETING

No comments were received.

3. SNBTS FFP SPECIFICATION (5)

The specification for fresh frozen plasma prepared by the Study Group,
had been discussed by the SNBTS Directors and suggested amendments had
been circulated to Group members, who agreed on the underlined changes.

1.01 Agreed that a separate specification for source plasma would be
prepared.

Also agreed a final sentence should be added to the FFP
specification regarding a regular (yearly) review.

2.01 Delete "individual."

2.02 Delete final sentence.

2.03 Add "except those derived from platelet rich plasma which should
contain at least 120 ml."

2.04 Delete final sentence.
2.05 Delete "to allow identification of donor(s)".

3.03 Change final sentence as follows: "... platelet count should be less than $30 \times 10^9 / \text{L}$ when measured in plasma packs representative of each week's production. A suggested sampling level is 1% per week."

3.04 Rework as follows:
"The methods of handling plasma following separation by centrifugation should prevent further microbial contamination so as to ensure a level of not more than 10 CFU/ml."

3.05 (a) Agreed that the words "prior to bulk freezing" should be inserted after "Appropriate arrangements should be made. .. " and deleted from the end of the sentence.

(b) The final sentence should be altered to read: "More than 70% should have a value of at least 0.7 u/ml".

This specification would be retyped and circulated by the HQ Unit.

4. FRACTIONATION UPDATE (6)

Dr Foster's paper "Progress Report for the Factor VIII Study Group" and the results/conclusions summarised therein provoked a full discussion of the study methods employed. In particular Tables 3A and 3B caught the attention of the Group, who noted the marked losses in FVIII:C and Factor VIII R:Ag between donor and frozen core samples of plasma. It was felt that these losses were undoubtedly due to the addition of citrate, either in anticoagulants or in process. Dr Prowse tabled a paper showing results obtained in follow-up studies of Mr Farrugia's work which seemed to confirm that citrate levels in donations could be reduced. It was agreed that these studies were of considerable significance and should be continued. Future studies would compare ionised calcium levels in samples obtained from machine plasmapheresis donors with those obtained from standard donor samples. Dr Foster should let Dr Prowse have plasma samples from batches received from the North East for testing in the same fashion. These samples should include plasma obtained by three different methods: (i) Haemascience machine plasmapheresis, (ii) hollow fibre machine plasmapheresis and (iii) routine donations. PFC staff should also check the citrate content of the anticoagulant used in manual and machine plasmapheresis bags and, once ascertained, should check the ratio at which each is mixed with blood.

It was clear, however, from the tables prepared by Dr Prowse that the stability of FVIII in the plasma samples could be improved. It was recommended that similar tests should be carried out using different methods of cryoprecipitation. Dr Prowse explained that information was already being collected on other plasma proteins and on the stored red cells from the donation already obtained.

Dr Dawes then explained the work currently underway to study the effect of citrate on white cells heparin release.
All of these studies seemed to confirm the previous conclusions regarding the effect of citrate content on plasma FVIII. Changes in downstream processing could not counteract the deleterious effect on plasma FVIII content, therefore the problem would require to be tackled at the Regional Transfusion Centres.

The following points for study were agreed:

1. Data to be collected on ionised calcium levels in plasma obtained via all the different types/methods of collection currently in operation.

   Dr Prowse would liaise with Dr Perry in this connection.

2. Dr Dawes to conduct basophil/heparin studies on 7-14 day old donations.

3. Following published work on elastase levels, the West had checked samples using anti-sera obtained from Germany, but no elastase had been demonstrable in ordinary plasma donations.

   Dr Dawes should liaise with Dr Gabra on this work.

4. Dr Foster should liaise with Dr Gabra in order to study platelet contamination levels in two batches of plasma obtained by machine plasmapheresis (Batches 798 and 4.004).

Discussion turned to Sections 2.1 and 2.2 of the Progress Report. As requested by the Group at its previous meeting Dr Foster had prepared a further split batch of lots NY771 and NY772. Unfortunately this had not clarified the previous study, the results being similar. No conclusion could therefore be drawn as to the accuracy of either the one stage or two stage assay.

Dr Boulton should pursue clinical evaluation studies with Dr Elizabeth Mayne (Belfast) but available product should first be dry heat treated.

A brief outline of the current collaboration with Professor Alan Johnson in the development of new processing methods was given. However, detailed information could not be made available as this work is covered by a confidentiality agreement. Information gleaned during this work is adaptable for use in the ZHT process but work on this had been suspended in favour of the collaborative project.

Dr Perry outlined the current factor VIII heat treatment programme. All non-heated product had been withdrawn and was now undergoing heat treatment.

Solubility of FVIII following heating had been improved with the addition of sucrose, allowing longer heating, and only a 20% loss of FVIII in finished product.

There were currently two batches which had been heated for 24 hours @ 68°F using the above method; clinical trials were now required.
It was decided that Dr Boulton should approach Dr Mayne with regard to the availability of Von Willebrand Disease patients who would be willing to participate in a clinical evaluation of this product. In particular the following parameters should be studied.

1. Whether product effective in VWD.

2. In vivo recovery and half-life.

3. LFTs to establish whether current heat regime inactivated NANB.

If Dr Mayne was unable to help, Dr Charles Rizza and Professor Arthur Bloom should be approached.

Clinical evaluation using virgin haemophiliacs would be reserved for future studies of high purity product.

5. VIRICIDAL OPTIONS UPDATE (7)

Dr Pepper summarised the points made in his paper which had been circulated. The options for future work had not changed since the previous report and future work areas had been delineated at items 2, 3 and 4 in the paper.

Dr Cuthbertson summarised the work carried out on the retrovirus group of viruses. Future studies would be carried out on both murine and feline leukaemia viruses. It was thought that the latter might produce results similar to HIV-III. It was agreed that the work delineated in the Lancet (Spire et al 1985 Lancet 188-1898) should be incorporated in these studies.

It was also agreed that the whole range of additives and viruses should be studied after freeze drying and heating; all of which would require to be timed to suit PFC production schedules.

Dr Cuthbertson reported on his recent visit to the Pasteur Institute in Paris and the work being conducted there on LAV inactivation. It was clear that this Institute would be willing to carry out tests, to detect HBV using their available DNA probes, if appropriate payment was made. However, no decisions were taken at the moment as it was thought that more data would be available over the next few months.

The final decision on whether or not the Group would reassess the recent irradiation data published by Margolis and Eissen was left to their discretion.

No further information had come forward regarding detergent inactivation from the New York Blood Centre. It was suggested that Dr Pepper should contact Celltech on this topic.

Chemical methods of virus inactivation remained a low priority for study.
6. FVIII ASSAY GROUP (8)

The minutes of the last meeting of the Group had been circulated. Dr Cash thought this was a good report on a successful exercise. All Scottish Centres except the North East had participated in the exercises; Northern Ireland have indicated they will join in. It was agreed that the Scottish QA exercise would be continued for another year, followed by a review of results obtained.

Dr Cash felt that the work of the group was worthy of publication. Opinions varied. Dr Prowse pointed out that a poster had been submitted for consideration by the Scotblood Organising Committee. After discussion it was agreed that Dr Gabra should prepare a first draft during the next few months with a view to possible publication in Vox Sanguinis.

Dr Boulton pointed out the current lack of data on assay values ≤10% and informed the group of Dr Christopher Ludian's activities in this area along with his possible need for artificial substrate. There was no question of the SNBTS making available quantities of substrate to non-SNBTS scientists/clinicians. The possible development of demand for this product was discussed and it was agreed that Dr Boulton should contact Dr Jean Thomson to establish current activities in Manchester - Dr Poller's laboratory were able to provide FVIII deficient substrate.

7. CLINICAL TRIALS OF HEAT TREATED FVIII (4)

Dr Boulton summarised the data contained in the letter/papers he had sent to Dr Perry on 21st January 1985 which had been circulated to the Group.

No real problems had been identified but it was agreed that more precise information regarding solubility should be included in future studies. It was also agreed that Dr Boulton should produce a "master" table of all results obtained in all patients, using different products.

Dr Perry was asked to inform the Group of the solubility problems which had been encountered with the use of filter needles, which would be coming on to the market along with the commercially produced heat treated FVIII.

Dr Perry outlined the information available from the West on heat treated product and agreed to let Dr Boulton have copies of this data for incorporation in "master" table (above).

It was agreed that Phase 2 product would require to undergo similar clinical trials. Dr Boulton should let Dr Perry have copies of protocol sheets for comment. Dr Cash agreed to write to Drs Ludian/Forbes and Mayne regarding Phase 2 product studies and that Dr Rizza and Professor Bloom should be contacted by Dr Boulton prior to the commencement of the study. Haemophilia Directors should be asked to keep serial samples from all patients receiving heat-treated FVIII. A protocol for a long term study including VWD patients, had been prepared by Dr Cash and would be submitted to the combined Haemophilia/Transfusion Centre Directors' meeting for consideration.

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8. (a) DETECTION OF HEAT INDUCED DAMAGE TO FVIII (3)
   (b) ANTIGENIC CHANGE IN HEAT TREATED FVIII (9)

   (a) Dr Pepper summarised possible difficulties which may occur as a
   consequence of heat treatment. The formation of aggregates and
   loss of biological activity would be dealt with at the quality
   control stage of production. However, Dr Cash thought aggregation
   should be investigated and Dr Pepper agreed to do so. Another
   possible problem could be enzyme activation: a chromogenic
   substrate assay should identify any activated enzymes. For in
   vitro tests paired samples with and without heating should be
   tested.

   There was a discussion of the value of obtaining patient data which
   would be useful for future high purity product. Serial samples
   could be obtained from patients to provide in vivo information;
   Fpa should be examined. Phase 2 testing could involve
   HTLV-III AB -ve patients (Dr Ludlam). It was agreed that Dr
   Perry, Dr Pepper, Dr Dawes and Dr Boulton would liaise on this
   project.

   (b) Dr Pepper explained current ideas and possible areas of study of
   neoantigenicity. It was possible that heat treatment incorporated
   sucrose into glycoproteins covalently.

   Dr Dawes was asked to repeat the radioimmunoassay parallelism
   study, excluding FPA.

   There was a question, also, whether Brenda Griffin should conduct
   tests of monoclonal antibody to FVIII Cag and FVIII Rag.

   Dr Prowse would pursue the question of electrophoretic analysis for
   VIII Rag multimer.

   In view of Dr Graham Bird's recent Lancet letter on the topic of
   antigenic change in FVIII it was agreed that Dr Cash would invite
   him up to Edinburgh to meet with the following who would form a
   sub-group to investigate this aspect:

   Dr Pepper      Dr Cash
   Dr Dawes       Dr Yap
   Dr Prowse      Mr McIntosh PFC

9. DATE OF NEXT MEETING

   Tuesday, 25th June at 10.30 am in the HQ Unit.

   Agenda

   1. Antigenic change in heat treated FVIII.
   2. Clinical trials - in vivo results (split batch).
   3. Ionised calcium in different types of plasma.