IN CONFIDENCE

MINUTES OF MEETING OF FACTOR VIII STUDY GROUP
Held in Headquarter's Unit, Ellen's Glen Road
on Thursday 12 January 1984

Present: Dr J D Cash (Chairman)
Dr C V Prowse (Secretary)
Dr F E Boulton
Dr R J Perry
Dr P Foster
Dr D S Pepper
Dr B Cuthbertson (morning only)
Dr G S Gabra
Mrs E Porterfield (Notes)

1. INTRODUCTION AND APOLOGIES FOR ABSENCE

Apologies had been received from Mrs B Griffin and Mr A Farrugia.

2. MINUTES OF PREVIOUS MEETING (2nd February 1983)

The following suggested amendments had been received from Dr Gabra.

3c page 3
Top of the Page
PFC felt that ... indicated by the tables.

to be followed by my comment which was not minuted namely. "The figure
in the tables were checked and are correct; ACD FFP that goes to PFC is
only around 1% and not 20% as mentioned by Mr Watt."

This is important because it leaves the members of the group with only
part of the information, which is also the incorrect part.

Same paragraph
The following sentence is also incorrectly expressed. It should read
"It was suggested by Dr Gabra in the document that QA of VIIIC:C in
FFP may preferably be assayed at one Central Laboratory, but the general
mood of the group was not – and it was agreed that each region performs
its own assays."

With these amendments it was agreed that the minutes represented a true record.

Before progressing to the reports received from the 4 Sub-groups Dr Cash felt
it might be appropriate to consider the future need for the Study Group.
He was of the opinion that the Group had contributed work of great value
to the SNBTS but wondered if it could now be wound up with the possibility
that one or two of the Sub-groups could continue to function, eg. Safety
Action Group, Assays and Standards Working Group. The possibility of
including /
including representation from the smaller Regional Centres and enlarging
the remit of the Group to include other coagulation factors was also
discussed but Dr Cash felt that in the meantime a Report of the Group's
work should be prepared for submission to the Transfusion Directors
who would then decide whether or not to change the remit of the Study
Group.

The opinion of the Group was that it should continue to meet on an annual
basis only, for updating purposes, except in the case of untoward events,
when an extraordinary meeting could be arranged.

It was therefore agreed that the next meeting would take place in the first
week of January 1985, the membership to be those in attendance on 12th

3. MATTERS ARISING

(a) Factor VIII Safety Action Group

i. Dr Pepper summarised the Report of 15 June 1983 which had been
circulated. In view of the time lapse between preparation of this
Report and the date of the meeting most of the data was now historical.
Heat inactivation of viruses still appeared to be the best choice.

ii. So far as other (non-heat) treatment was concerned Dr Pepper had been
unable to ascertain the method employed by Immuno but would continue
to pursue this. Both he and Dr Cuthbertson planned to conduct more
radiation experiments but these were of a low priority.

Organic solvents were more popular in USA since they act by physical
rather than chemical actions but the position regarding licencing was
unclear.

Local tests on Hyate concentrate irradiating with 2.5 M Rads at
different temperatures were intriguing but resulted in unacceptable
loss of solubility.

It was agreed that Mrs Griffin would make up a batch of highly pure
concentrate (fibronectin and fibrinogen) to conduct further tests. If
it could be freeze dried, it could also be tested with the addition
of carbohydrates. There was the possibility that this technology could
be relevant to FIX. Dr Cuthbertson informed the Group that he hoped

to
to have some results on this work in six months' time. Discussion moved on to chemical/detergent inactivation. Dr Pepper recommended that the effect of hydrogen peroxide on clotting factors ought to be studied. This was potentially quite harmless as it broke down to water. Dr Perry was unsure of the possibilities of reducing such an addition to production practice. Dr Pepper agreed to look at the addition of hydrogen peroxide but was uncertain that it would inactivate hepatitis B.

iii. AIDS

The position was well known. High risk donors were dissuaded from donating. Dr McClelland had prepared a report following a WHO meeting in Geneva in November 1983.

Dr Pepper summarised the work on HPLC assay of the GTP metabolite neopterin. It is claimed this assay accurately segregates early AIDS from other patient groups. However it would not identify potential victims, merely existing victims. Further work on high-risk groups is awaited.

Dr Cash would ask Dr Dawes to follow up this work with a view to a possible study.

iv. Hepatitis B

Considerable progress worldwide noted on new probe for Hepatitis B-DNA. It was felt that SNBTS should have someone familiar with this technology and it was agreed HQ Laboratory should pursue and investigate equipment costs etc. Any new technology etc developed in HQ lab would be available to the SNBTS as a whole.

Dr Cash proposed, in view of the fact it had been decided to meet annually in future, that there should be ongoing regular meetings between Dr Foster, Dr Perry and Dr Pepper on this general topic. However, Dr Foster was quite happy with the current links between Dr Cuthbertson and Dr Pepper.

Dr Cuthbertson had been invited to enlarge on the section of the Report from Dr Foster entitled "The Effects of heating on FVIII C and Model Viruses", as the work was conducted as part of the Safety Sub-Committee. Dr Cuthbertson had been working since early 1983 with Dr Alex MacLeod of PFC to study the effects of heat on the inactivation of viruses in FVIII (prepared by zinc fractionation method) and in PFC albumin (with caprylate), stabilised with either Sorbitol/glycine or sucrose/glycine. Behringwerke sucrose/glycine had been used as control protectant solution.

Various /
Various slides showing results were shown which are summarised below:

1. **Inactivation of Vaccinia at 60° in albumin – various stabilisers**
   
   These studies compared ordinary SPPS (caprylate stabilised); SPPS (sorbitol/glycine stabilised) and Behringwerke (sucrose/glycine stabilised) 7.5 log, 3-log and 4-log reduction, respectively, in virus titre were found.

2. **Inactivation of Vaccinia in Sorbitol/glycine stabilised albumin heated to different temperatures**
   
   By raising the temperature 10°, inactivation of virus was achieved in a much quicker time. This could be applied to product by heating for 10 hours at 60°C then incorporating a 70°C stage which gives compatibility with previously used methods, although a similar inactivation is achieved in only 45 min at 70°C. Such treatment allowed a 7-log reduction in virus titre.

3. **Inactivation of Vaccinia in Sorbitol Stabilised FVIII**
   
   In this study one test was conducted on product heated to 70°C after 9 hrs 15 mins at 60°C and one test on product heated to 70°C for ½ hr. While the latter gave better results the former is preferred as it allows incorporation of established procedures and allows 75% retention of factor VIII with a 6-log kill or more.

4. **Inactivation of Herpes Simplex**
   
   Provided the following results:
   - SPPS Control: no virus left after 60°C for 10h.
   - Sorbitol/Factor VIII: little virus after 60°C for 3h.
   - Sorbitol/Factor VIII: no virus after 70°C for 15 min
   - ie 6-log kill or more

5. **Inactivation of Polio 2** (non-enveloped, RNA virus)
   
   Results were:
   - SPPS Control: no virus after 60°C for 10 h (ie 6-log kill or more)
   - Sorbitol/Factor VIII: little virus after 3-6 hours at 60°C
   - Sorbitol/Factor VIII: no virus after 15 min at 70°C
   
   Therefore the only virus of these three which is not inactivated when heated to 60°C is vaccinia, which required a period at 70°C.

6. /
6. Inactivation of Mumps

Published data suggested inactivation of this virus should occur at 55°C. Studies conducted revealed the following:

- SPPS: heated to 60°C; very little activity remains after 30 mins (i.e. greater than 5-log kill)
- Sorbitol/FVIII: heated to 60°C: $10^5$ reduction at 10h
- " 70°C: $10^3$ reduction at 1h

It was concluded that the mumps inactivation studies required follow-up.

Dr Cuthbertson summarised possible future work:

1. In the next experiment with mumps virus, the incubation period would be extended to 22 hours as evidence points to the fact that this does not affect factor VIII.

2. a. Work conducted by Dr Smith BPL suggests there is no yield penalty for dried FVIII if it is heated to 60°C for 3 days. This would be investigated, but it was noted that the current Hyland product made by this method is still ineffective.

b. Option to freeze dry in HQ Lab drier, but it would be difficult to control water content. This is considered merely as a back-up option.

Dr Cash thanked Dr Cuthbertson for his contribution.

(b) Assays and Standards WP

Dr Prowse summarised the Workshop held in March on the topic of the SNBTS standardised assay for FVIII which had been attended by staff from Dundee, Aberdeen, Edinburgh and PFC.

The results from the first FVIII QA exercise, which were promising, were summarised. Aberdeen (NE Scotland BTS) had not participated in this exercise.

The second exercise was in progress. Dr Prowse undertook to let Dr Cash have the final results of these two exercises when completed.

It was agreed that the final report from the Study Group to the Transfusion Directors would recommend that an SNBTS Factor VIII QA group should be established.

In /
In the meantime once the results of the second QA exercise were known it would be decided whether or not to continue the meetings of the Assays and Standards WP, but it was thought that possibly a meeting twice/year would be necessary to keep work going.

Discussion turned to the topic of chromogenic assays for which a new kit would be available shortly. This assay currently caused some problems, especially when carried out manually but these were being studied at the moment.

It was agreed that Dr Prowse would ask Kabi for further kits in order that inter-laboratory assessment could be conducted during the next QA run, if possible. If necessary Dr Perry agreed to investigate purchase of such kits for this exercise. The question of adapting ELISA equipment to carry out chromogenic assays (in those centres without spectrophotometers) was discussed.

Dr Prowse summarised the studies conducted on FPA levels which seemed to indicate that the mode of mixing was not of great importance but that other parameters such as the stripping of blood lines into bags had some effect. It was agreed that further investigations in this area be carried out by Dr Gabra following discussion with Dr Prowse.

A review of "From Donor to Fractionator: How Factor VIII is Lost" had been prepared, as requested at the previous meeting, and was tabled.

(c) FVIII Yield WP

2.1 Dr Foster asked Dr Prowse to enlarge on the results of the work done by Albert Farrugia on the influence of calcium on FVIII:C; these are shown in the table "Stability of FVIII:C in plasma of various Ca\(^{++}\) concentrations". This study would seem to indicate that the addition of calcium to CPD plasma may assist in the prevention of loss of FVIII:C during liquid storage.

It was agreed that this work looked promising and was of a high priority but, in view of the fact that Mr Farrugia would not be available for much longer, it was necessary to obtain the services of another scientist to continue this line of work.

2.2 Adding Calcium to Standard Process

Follow up work since last meeting was summarised; the results for a split batch (with and without calcium) are shown in tables on page 2 of Dr Foster's report. A similar discrepancy between one-stage and two-stage assays had previously been reported by Dr Rock and it was thought /
thought that the only way to resolve this problem would be to conduct in vivo studies. A quantity of each batch had been retained for this purpose should it prove possible.

It was unfortunate that in this case only a 10% difference between the two batches of FVIII had been demonstrable. Typically a 20% improvement had been noted.

It was agreed that, in order to confirm any benefit of calcium addition, PFC would produce another split batch for in vivo testing. The following approaches would be made.

(a) The remaining 20 vials of NY771 and NY772 would be sent to Dr Prowse for assay using various methods.

(b) Dr Boulton would ask Dr Bruce Bennett, Haemophilia Director, Aberdeen to help and also ask Dr Ludlam if he would participate in small scale studies. Dr Charles Forbes, Glasgow, would also be approached.

(c) Dr Cash would let Dr Foster have the details of the amounts required for in vitro studies soon.

(d) One patient had reacted adversely to administration of product from batch No NY761 (heat treated). It was queried whether this may have been due to sorbitol. Dr Boulton was asked to investigate the clinical use of sorbitol solutions and report back.

Newer batches had lower sorbitol and lower zinc levels.

If the same patient was agreeable he could receive some of the product heated to 70°C. It would also be useful to know if product heated to 70°C was suitable for von Willebrand patients. If possible it would be helpful to have LFTs carried out in a virgin haemophilic group. Possibly this could be done in Oxford. Dr Cash agreed to approach Dr Rizza about this. It was hoped that a limited supply of heat-treated factor VIII would be available for routine clinical use from late Autumn 1984.

3. New Processes

3.1 Zinc Fractionation

Scale up studies have emphasised the importance of careful mixing during zinc addition.

3.2 Heat Treatment

The work being carried out on effects of heat on the inactivation of viruses had already been summarised by Dr Cuthbertson.
3.3.1 Ultra Filtration

Because of problems which Dr Foster outlined for the Group work had been commenced on precipitation as an alternative.

3.3.2 Precipitation

The results to date were encouraging. During experiments it had been possible to precipitate different heated Factor VIII solutions such that there was no detectable VIII in the supernatant.

When re-dissolved as much as 80% of the Factor VIII:C had been recovered. The glycine/sodium chloride precipitation also achieves further purification: since protein and zinc are removed.

3.4 Neo-Antigens

Dr Dawes and Mrs Griffin have been involved in carrying out a range of radio-immunoassays comparing heated and unheated material. Dr Foster showed a poster illustrating the standard dilution curve of Factor VIII:C; Factor VIII R:Ag, Fibrinogen, PF4, thrombospondin and β-thromboglobulin in FFP and Factor VIII concentrate with and without heat treatment for 10 hours at 60°C. In all cases curves for concentrate were parallel to those for plasma indicating no detectable change in antigen structure. Only FVIII R:Ag had so far been assayed after heating to 70°C.

(d) Quality of Plasma WP

Discussion was incomplete when the meeting adjourned; to be reconvened on Tuesday 14th February. Proposals for amendment will be circulated after that Meeting.