A. FARRUGIA - OXFORD PFL VISIT ON 9TH AND 10TH JUNE 1982

Discussions with Jim Smith

1. INTERMEDIATE PURITY VIII CONCENTRATE: Present process gives a yield of \( \simeq 280 \text{ ug/kg, sp. act. } \simeq 0.4 \text{ umg} \) and on dosage 14 - 17 u/ml in the dispersed material. Went over the history of the cold precipitation - introduced because previous concentrate gave filtrability, solubility problems. Cold ppt, which is obtained as in the published work, gives a substance which is strongly adhesive and highly insoluble. J.S. says that this stuff contains 40 - 50% of the original plasma fibronectin and he is convinced that previous opinions that fibrinogen is the cause of filtrability and solubility problems are wrong - fibronectin is the culprit. They are working on the cold precipitated material with the aim of making a clinical C1g rich product, but have great problems in obtaining a product that will freeze dry and solubilise well. Re-improving their VIII yields in concentrate, they have a continuous thaw tank set up and hope to start a process similar to the P.F.C. one soon; their present low yield is mainly because of a high loss in the Sharples during the cryoprecipitate sedimentation.

2. HEPARIN: They have looked into Gail Rock's claims - assaying VIII in heparinised samples also proved a great problem. Protamine titration using the A.P.T.T. did not work, but using the 2-stage assay worked by apparently adsorbing heparin on the alumina. Recently they have started using a 1-stage assay based on a prior titration using the N.A.P.T.T. - this gives better results than the A.P.T.T. titration. Using these methods they have found (1) no difference in VIII:C levels in plasma collected into CPD, heparin, or CPD/heparin. (2) no difference in VIII Cryo yields with heparin in the plasma at the levels Rock gives, but an altered protein composition in the Cryo at heparin levels in the CPD plasma of 15 - 20 u/ml.

3. PLASMA QUALITY AND HARVESTING: J.S. maintains no difference in final yields in VIII int. purity concentrate with 6 and 18 in old plasma, except in
plasma from one centre (did not say which) which he suspects is freezing its '6 hr.' material much earlier. He has found very significant differences in VIII yields between ACD and CPD plasma - starting levels are higher in CPD, yields are higher and cryo is different - Cryo from ACD needs a higher temperature to extract fully the VIII. The difference in VIIIc levels and process yields in different blood groups has been noted by them - he says that for some reason the differences observed in Oxford are even higher than the published data (i.e. of the order of 0.7 u/ml O plasma to 1.3 u/ml A). He has the impression that the difference becomes more accentuated the more one processes i.e. 0 < A in the plasma yields: 0 < A in the Cryo O < A in the final product. Has found no difference in his final process yield between plasmas frozen at different rates.

Packs are opened in two ways:- Liquid Nitrogen Cracking (single donations) and tearing after alcohol swab.

4. BIOCHEMICAL ENGINEERING: I asked about the work being done in Oxford. He seems to be abreast of what's happening and is not very impressed.

His opinion goes something like this: "Even if they get it into the bug, which they are a long way from doing (problems from the nature of VIII - carbohydrate, heterogenous molecule etc.), they are going at best to end up with a room-sized tank of a very dilute, very dirty, solution which will need cleaning, concentrating etc." So the problem will end up with the fractionators with their expertise on these problems.

5. OTHER PROCESSING METHODS: Polyelectrolytes, according to him, have not proved very good "they give 40% of what we start with, which is cryo-precipitate (at 40% yield from plasma) and the purity is not improved. However, he hinted that the aminohexyl chromatography method of Dennis Austen has been further looked into and (they have something which will soon be published) has given good results. But he was a bit reticent about this.

6. OTHERS: Went over the FIX process - DEAE cellulose on diluted cryosupernatant product fails the Tg₅₀ but is safe in animal tests. Once a month they
make FVII concentrate which is mostly used for four congenitally
deficient patients in the Oxford area. They are developing an ATIII
concentrate which is obtained in good yield after pasteurisation - J.S.
is not convinced that the German process for pasteurising FVIII is
removing totally the infectivity and sees the low yields as being totally
unacceptable, but thinks that upping the yield to 20% would be a
worthwhile target.