0016

Scottish National Blood Transfusion Service
Protein Fractionation Centre,
Ellen's Glen Road,
Edinburgh,
EH17 7QT

Tel. Nos.: 031-664 2317
Director: GRO-C
Telex: GRO-C

Scientific Director:
John G. Watt

Our Ref: prf.ww
Your Ref.

20th January, 1983

Dr. J. K. Smith
Plasma Fractionation Laboratory
Churchill Hospital
Oxford

Dear Jim,

We are still spending most of our time studying heat treatment of FVIII but since your last letter Alex has repeated the FIX experiment with similar results to those I sent previously (65 - 70% recovery). However in both of these experiments the pH was uncontrolled and fell to approximately 6.7 after the addition of glycine. Because of this Alex has also carried out the experiment with the pH held at 7.2 (using sodium hydroxide). In this instance the recovery after heating fell to about 30% and we therefore suspect that pH or salt content may be crucial. All of this has been done with a common source of FIX (a pyrogenic batch of DEFIX) and there is the possibility that marginal changes in salts may be important. We will have to look at this more seriously. Our pyrogenic batch of DEFIX is virtually all used-up but there is quite a lot of DEFIX on its way through process & we are waiting to see if any batches fail Q.C. If something does fail we will send some to you, but if not we may have to 'steal' some of the genuine article. This would normally be O.K. but could be difficult at the moment because of a number of factors:-

a) We will be stopping production in early February for about one month to bring the Centre closer to M.I. standards.

b) There is growing interest in supernine (at about 30-40% yield from DEFIX).

c) There is growing interest in DEFIX for use with FVIII inhibitor patients.

As soon as I find out what we can manage to send I will let you know. If you need more details on the heat studies I suggest you contact Alex directly.

The FVIII project is progressing well we are consistently seeing 70-80% recovery (1-stage assay) over the heat step but there are major losses during the addition of sorbitol and we have to do a lot more work on removal of the stabilisers. We have noted some crucial points. The pH must be controlled during glycine addition and during pasteurisation; sorbitol has a -ve heat of solution and we think the problems associated with the addition of sorbitol may be resolved by maintaining and increasing the temperature according to sorbitol concentration.

I'm/

I'm not sure that our observations really differ on the citrate-mediated inactivation problem. Our initial results were from process solutions using 0.02M citrate. We then attempted adjustments in-process which caused headaches such as poor solubility which I mentioned in my last letter. After that we retreated into the lab and investigated various reagent formulations. We did this by taking solutions from process (prior to salts addition), adding various reagents, leaving the solutions on the bench for 2 hours then freezing and freeze drying. Controls were built into every experiment (i.e. no salts and 0.02M citrate). We are now close to finishing the project and from an initial look, the data confirms the previous in-process results (i.e. 20-30% loss in 0.02M citrate). This loss is substantially reduced at lower citrate levels (0.01M & 0.005M) but we have seen problems with filtration, solubility and even clotting at the lower citrate concentrations. I now wonder if perhaps the different experience between PFC and PFL/BPL vis-a-vis the need for a cold-step may be relevant here.

We have also found the citrate-loss to be associated with ionised calcium levels and that the loss can be prevented by adding small amounts of calcium. Obviously the major improvement is with 0.02M citrate but addition of calcium also improves the lower citrate groups. These results were more pronounced in the freeze dried sample suggesting that frozen samples may be a little more labile (perhaps due to thawing prior to assay). FpA assays have been carried out to see if calcium addition caused any activation, but the early results (n=8) actually show a significant reduction in FpA after calcium addition. Other substances that we have looked at include glycine, heparin, phosphate and maltose. Glycine gave poor results but the phosphate recipe (Liu et al Vox Sang. 38, 216-221, 1980) is looking very good. Maltose does have a marked effect on solubility and we are currently comparing this with dextrose.

The next step will be to move back into process and test the chosen option for real. From the lab data so far the best solutions seem to be 0.02M citrate + 3μM calcium or phosphate/NaCl and I suspect the phosphate option may be preferable philosophically. Long term stability data will also be crucial.

After this the remaining source of loss (apart from plasma quality) seems to be during aluminium hydroxide adsorption. How vital is this for stability (rather than filtration)? - I seem to remember that Hyland get away with heparin instead!

The supernine trial has not yet addressed the hepatitis problem. The studies to date have covered FIX recovery and thrombogenicity and as far as we know recovery is normal and there is no evidence of thrombogenicity (no rise in plasma FpA). I think it has been generally agreed that there are not enough patients to test the hepatitis situation properly and chimps were being thought of again. However, all this has been overtaken by heat treatment and there seems to be a view that pasteurisation is philosophically preferable to PEG fractionation (regardless of yield). All we can say at the moment is that the options seem to be growing and ideally we would want to know all the various hepatitis/ thrombogenicity profiles. Because of point (c) above it does look as though DEFIX may be retained, at least until we have a proper solution to the inhibitor problem.

We are very pleased with out progress with I.V. IgG. We seem to have a good product according to the in vitro tests and clinical testing is now underway. One of the biggest difficulties was sorting out various problems with both the anti-complementary and PKA assays.

Work/

Work with antithrombin III is progressing a little more sedately and we are currently putting effort into deciding appropriate quality control procedures and in learning how to cope with soft gels at scale.

I'm interested in your method for preparing thrombin (if its not classified). There has been no suggestion that we should prepare this (and I wouldn't want to encourage it just now) but it would be nice to have something on file just in case it ever comes along.

Best wishes.

PETER R. FOSTER

P.S. BTG have been looking at the patent situation with sorbitol and have decided to try and file something.