PROPOSAL TO DEVELOP A "HEPATITIS-SAFE" FACTOR VIII CONCENTRATE

1. Introduction

Factor VIII concentrates, in common with many other coagulation factors, are implicated in the transmission of at least three diseases:

Hepatitis B; transmitted by a virus with well documented marker antigens and antibodies.

Non-A non-B hepatitis (NANBH); thought to be transmitted by a blood-borne virus, but for which there are still no reliable markers.

Acquired immune deficiency syndrome (AIDS); not yet proven to be of viral origin, but this is strongly presumed.

The incidence of hepatitis B is diminishing, possibly because haemophiliacs receiving many batches of large-pool concentrates become immune, and partly because of improved screening of the plasma used for fractionation. The incidence of NANBH, especially on first treatment of mildly affected patients, remains very high and screening cannot yet be applied for want of markers. NANBH causes increasing concern, less on account of its acute effects (although deaths have been reported) than because of its association with chronic active hepatitis in later life.

Factor VIII coagulant activity has always been regarded as exceptionally labile, and it is only recently that serious attempts have been made to apply to factor VIII concentrates some physical and chemical processes designed to inactivate hepatitis viruses. As with other concentrates, the options open to fractionators (excluding screening and vaccination) are:

(1) Immunological neutralisation or immunoadsorption on solid phase antibody.

(2) Physical removal of infective agents by e.g. semi-specific adsorbents or precipitants.

(3) Inactivation by heat or virucides.

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2.1 Immunological methods

Tabor et al have demonstrated a reduction in transmission of hepatitis B by factor IX concentrate after adding a small amount of high-titre human antibody. It is known that some companies have tried immunoadsorption on solid phase anti-HBs but these have been animal antibodies, carrying some risk of leakage and inadvertent immunisation of patients; factor VIII is susceptible to inactivation during the long periods of contact required for efficient adsorption; and proof is lacking that even a specific antibody with very high affinity can remove viruses to below the infective level.

There is no convincing report of the removal of NANBH virus and it is likely that a number of antibodies would have to be used, in either the neutralisation or adsorption mode, to affect the risk of transmitting hepatitis.

This approach does not, therefore, recommend itself at this stage in our knowledge of infective agents.

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2.2 Physical removal

At least two non-specific adsorbents, polyelectrolyte E5 and amino-hexyl sepharose, bind factor VIIIC so tightly that adsorption of a crude factor VIII concentrate can be followed by extensive washing, reputedly sufficient to elute poorly-adsorbed hepatitis B markers. The stage yield for such processes, which also tend to separate VIII vWF from VIIIC, is of the order of 40% and no evidence has been offered for the 4- to 8-log reduction in titre required to remove infectivity, even using hepatitis B markers.

It is plausible that the PEG precipitation used by some manufacturers to remove less soluble contaminants from factor VIII also precipitates some viruses, but removal is certainly incomplete since transmission rates with such concentrates remain high. The solubility of factor VIII in PEG solutions leads one to predict that this method is unlikely to be modified to give the desired separation of factor VIII from viruses.

2.3 Inactivation

2.3.1 Virucides

The chemical agent most commonly used to sterilise proteins is β-propriolactone (β-PL), usually applied prior to UV irradiation, their lethal effects being additive. No successful sterilisation of factor VIII has been reported by Biotest, the company most interested in this approach. The carcinogenicity, mutagenicity and explosiveness of the reagent, and the difficulty of controlling both the chemical and physical parts of the treatment, make this an unpromising route at the moment. Other virucidal agents, such as hypochlorites, glutaraldehyde and ethylene oxide used to sterilise apparatus, appear to be highly denaturing in the concentrations used for sterilisation and their effect on hepatitis viruses is inferred rather than proven. Little has been done to investigate the selective denaturation of viral nucleic acid by methods which do not affect proteins; nucleic acids seem to be more resistant than proteins to most lethal treatments so far devised.

2.3.2 Heating

There is substantial experience in heat-inactivation of these viruses, particularly HB, and some of the potential pitfalls have been described. Whereas heating albumin (protected by addition of e.g. octanoate) at 60° for 10h reliably destroys all known hepatitis viruses, the same treatment may not suffice to destroy virus in all media. Even brief boiling at 100°- may be ineffective. Any agent added to improve the resistance of the protein to denaturation may also improve the viability of the virus whose destruction is intended. However, heat-treated albumin has a long record of perfect safety (provided the minimum parameters are achieved) and pasteurised AT III has been successfully tried in chimpanzees. Pasteurisation methods and additives have been described for plasminogen, factor XIII and IgG.

It is reported that, on theoretical grounds, amino acids such as glycine should protect polar groups and sugars such as sorbitol or sucrose should protect non-polar groups in proteins during heating. In practical pasteurisation experiments, at least partial protection of factors II, VII, IX, X, XIII and fibronectin has been confirmed. At least two commercial fractionators are claiming a reduced hepatitis risk from "heated" factor VIII concentrates and enough information is emerging to begin to repeat and extend this work.

Heating is considered the most promising approach to virus inactivation because:

(1) It is likely to be of broad application i.e. conditions which inactivate the exceptionally robust HB are likely to inactivate other blood-borne viruses.

(2) The treatment is cheap, relatively easily controlled, recorded and scaled up with precision.

(3) Extensive experience with other successful pasteurised proteins such as albumin offers readier regulatory and clinical acceptance than the use of a novel or unfamiliar chemical virucide.

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2.3.3 Irradiation

As well as UV irradiation noted above, there has been slow progress with sterilisation by ionising radiation. At the moment, irradiation sterilisation of animal feedstuffs and, in some countries, human food is allowed, but no instance is known of the sterilisation of protein solutions intended for intravenous use. Selective protection of proteins against ionising radiation may be more difficult than protection against thermodynamic effects.

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2.4 Fractionation of plasma from small panels of "accredited" donors

The inactivation of hepatitis viruses, e.g. by heating, may not be achieved rapidly for all proteins by a method giving an acceptable yield or may be delayed by some other aspect of product quality or safety. Fibrinogen may prove to be exceptionally difficult and progress even with factor VIII may be uncertain. It is therefore worth pursuing an alternative low-technology approach, the fractionation in wholly sterilisable equipment of small pools of exceptionally well screened plasma. The criteria for screening will vary with the virus in question, but the "accredited donor" system operated at North West Thames RTC would be a good example.

Schemes for comprehensive small-pool fractionation have already been proposed for the new BPL, and some of the possibilities are summarised again here:

12-donor pools of cryoprecipitate	Factor VIII, fibrinogen
Cryosupernatant from about 200 donations	
"Fraction I" precipitation	Fibrinogen, factor XIII
Adsorption with DEAE-cellulose	Factors IX, II, X, IIa
Adsorption with DEAE-sepharose	Factor VII
Adsorption with heparin-sepharose	AT III, factor XI
Cohn fractionation to fraction V	Unmodified albumin

The economics of small pool production vary with the demand for each protein and what other proteins have to be recovered. Theoretical options for recovery of individual proteins are appearing continually from the development programme, but at some point it may be necessary to put them together in a practical scheme for firmer evaluation of costs.

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3. Development of Option 2.3.2, Inactivation of Virus by Heat

3.1 Existing methods used to protect individual proteins during pasteurization have been developed empirically and explained later (if at all) by reference to the structure of the protein. Where so little is known about even the active centre(s) of factor VIII, not to mention requirements for the stability of the coagulant and carrier proteins as a whole, it is unlikely that a protective agent can be 'designed' by theoretical observations.

Although it carries obvious risks of over-commitment to certain avenues of enquiry, we will need to focus on what little empirical knowledge we have in order to reduce the infinity of theoretical options, e.g.:

nature and concentration of additives;
rate and temperature of adding additives;
pH;
ionic constitution of medium;
degree of purity of factor VIII before heat treatment.

3.2 The effects of these and other variables must be evaluated against such interactive criteria as :

yield of factor VIII (intermediate and finished for clinical use);
cost of further treatment required (process time, labour, equipment);
stability of heated factor VIII, and other quality and safety considerations.

3.3 Our present information is based on :

a) Behringwerke's process;
b) information, confidential at the moment, from PFC, Edinburgh;
c) limited experience at BPL and PFL.

To prepare a successful product, the following stages appear to be necessary :

i. prepare an adequately stable 'intermediate' factor VIII e.g. from cryo extract (3.3.1);
ii. remove or greatly reduce fibrinogen in the intermediate (3.3.2);
iii. prepare factor VIII for passivisation by ionic adjustments and additives (3.3.3);
iv. pasteurize (3.3.4);
v. remove additives (3.3.5);
vi. finish as freeze-dried factor VIII (3.3.6).

3.3.1 Preparation of Suitable Intermediate

It is likely that current methods of producing cryoprecipitate, and the programme of further improvement in cryoprecipitation, will offer a

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suitable starting fraction from FF plasma. In the first instance, it will be assumed that extraction of cryoprecipitate in a small volume of dilute tris buffer is favourable. The options of adding an adsorption stage (improved stability with little albumin yield) or a cold precipitation stage (removes fibronectin at a cost of 10-20% yield of factor VIII) will be assessed against the processing time required.

3.3.2 Removal of Fibrinogen

If fibrinogen is not removed or substantially reduced before pasteurization, it denatures as a solid or spongy mass which entraps a large proportion of factor VIII-rich liquid. Among the methods proposed for potential large scale separation of fibrinogen from factor VIII with a differential solubility are :

a. ethanol-glycine-citrate solutions;
b. polyvinyl pyrrolidone;
c. glycine;
d. polyethylene glycol (PEG)
e. Zn^{++}.

Some of these methods offer at least partial recovery of fibrinogen as a useful by-product and some give a useful fibronectin-rich fraction, rather than condemning them to 'nuisance' status.

On the basis of in-house experience with fibrinogen precipitation, methods based on zinc precipitation and on glycine precipitation (possibly combined with brief heating) will be preferred in the first instance.

3.3.3 Preparation of Factor VIII Solution for Pasteurising

It is expected that pH, ionic strength, concentrations of individual ions and protein concentration will influence the recovery of factor VIII on pasteurization and will have to be optimised empirically. Effective pH will change greatly during pasteurization and may need to be offset beforehand by additional buffering of titration.

From information so far available, the most favoured protected agents are glycine (which in some variants for the removal of fibrinogen may already be present in the solution) and a sugar (e.g. sucrose or sorbitol) in combination. It is said that the method of adding these reagents, in the exceptionally high concentrations required to protect factor VIII, brings its own problems and may need much attention during scale-up.

3.3.4 Pasteurization Conditions

It will be assumed that criteria for 'pasteurization' of proteins, in the hope of inactivating hepatitis B virus, are the same as for albumin, namely holding at 60 ± 1^{0} for 10h, irrespective of warming and cooling periods. Efficient heat exchange in very viscous solutions may need some assistance from chemical engineers.

3.3.5 Removal of Additives

It will be necessary greatly to reduce the concentration of additives, and perhaps change pH and ionic strength as well. Some options for this stage are :

a. group separation of HMW and LMW solutes by gel filtration. This approach can give very precise and efficient removal of additives from protein, but does not offer reconcentration of factor VIII

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and will certainly be impeded by the viscosity of the solution.

b. diafiltration/ultrafiltration. This is the most promising method, since complete removal of solutes is unnecessary and since the same membranes permit solute exchange and reconcentration of the protein. The cost of scaling up would be very high and there are potential problems in re-using the expensive membranes from batch to batch;

c. readsorption of factor VIII and selective elution. No adsorbent is known which will select factor VIII from the predictedsolution in good yield, but polyelectrolytes and aminohexyl-sepharose might be tried if (a) and (b) prove to be inappropriate.

3.3.6 Finishing

Sterile filtration of pasteurized factor VIII should not be difficult, provided stages 3.3.2 and 3.3.5 have been carried out effectively, but if the factor VIII emerges in a rather pure or dilute condition, it may be necessary to develop stabilizers to protect factor VIII during freeze-drying, dry storage and after resolution; and to improve the solubility of the freeze dried powder.

4. Project strategy and budget considerations

I anticipate that one of two likely patterns will emerge from the present flurry of activity. Pasteurisation of suitably protected solutions will give a concentrate safe enough for clinical trial and in a yield sufficiently promising to envisage scale-up to the "national product"; or we will know within about six months that the problems of pasteurising will need some random breakthrough or take several years to resolve.

I hope to draw up a more detailed outline of work, sharing but also overlapping with PFC's programme, during February. Until approximately April/May, empirical work among the most promising protective agents will continue under Rosemary Baker at BPL, at negligible material costs.

By April/May we may have been sufficiently encouraged by this approach to begin tackling the processing problems at PFL, while assisting RB with her continuing study of the chemical variables, if necessary. If progress were rapid, I would then suggest a fairly intensive four-month programme aiming at batches for clinical trial in October, even if some problems of yield, scale or efficiency remained. In order to achieve this, some of the present alternatives for factor VIII processing would be pre-empted and this project would receive the maximum possible attention at PFL from myself, David Evans and one or two selected development staff. I would negotiate with Dr. Snape for an appropriate support service from PFL Control. Particularly since diafiltration/ultrafiltration is the most promising option for solute removal, an infusion of about 10,000 may be required during the year 1983/4 for purchase of pilot ultrafilters, membranes and cartridges, hygienic production hardware, etc. I am reviewing what spare ultrafiltration capacity might be available at BPL in order to reduce the pilot-scale costs, but there will be no escape from a substantial commitment when production proper begins. It is possible to view such investment as offering hopes of a more concentrated product with substantially reduced capital and revenue costs for freeze-drying plant in the new BPL. There are precedents for seeing such purchases as advance investment.

If severe obstacles are encountered during the period April-June, it will be prudent, while maintaining efforts on pasteurisation, to initiate a second front. I suggest that this should be in the field of chemical virucides, the other area offering hope of general application. Since R and D Department is not well endowed with scientists who think naturally in biological terms, BPL would need either to marshal its staff resources from other departments, possibly to the extent of providing a project leader from Quality Control Department, or provide (say in autumn 1983) space and a new establishment for a biologist with a biochemical bent.

Two other factors will influence the speed and cost of development; the extent to which we can share information with allies and co-belligerents (e.g. PFC and Red Cross in Europe) and attitudes outside BPL to the question of clinical trials versus the use of chimpanzees.

I would like to have a very free hand, especially in these early months, to avoid a duplication of effort with other trustworthy laboratories. Since we start from some way behind, a modest stance will be quite appropriate. We should also envisage the possibility of having to use, and pay for, proprietary information e.g. through a licence arrangement. Especially if we prove to be protecting virus as well as factor VIII during heating, it may e.g. be necessary to replace or combine heating with a more specific virucidal treatment. Such a random solution (see e.g. Shanbrom reference) could emerge anywhere at any time. The Production Department should, therefore, perhaps budget for possible royalty payments in 1984/5 while hoping that in-house work will save these costs.