17 AUG 1976

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17th August, 1976.

Dr. W. d'A. Maycock,
Blood Products Laboratory,
The Lister Institute,
Elstree, Herts.

Richard
This dft of paper may interest you. I must admit to not having read J.K.S's paper v. clos

Dear Dr. Maycock,

Dr. Bidwell has asked me to draft some thoughts on a more consistent programme for exploring better factor VIII concentrates. The enclosed memo may have to be expanded later, but it should indicate the kind of minimum product I would aim at and some of the potential means of getting there, without giving too many specific hostages.

GRO-C

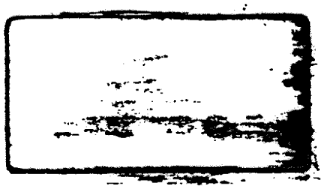
Yours sincerely,

GRO-C

James K. Smith.

13-280





357

## Partial programme for improvement of factor VIII concentrates

Influence of anticoagulant, methods of separation, freezing, storage and thawing on (a) FFP factor VIII and
(b) recovery of factor VIII and contaminating proteins in cryoprecipitate.

Methods of sampling and assessing factor VIII content of 5 litre packs and pools of FFP.

Influence of time, temperature, pH and solvent on selective extraction of factor VIII from cryoprecipitate, and consequences for subsequent adsorption with Alhydrogel.

Effect of additives on factor VIII activity and solubility of SIRV after sterile filtration and after freeze drying.

Influence of pH, titrant and protein concentration on cryoprecipitation of SIP and SIRV.

Maximum recovery of factor VIII from SIP, SIRV and cryoprecipitated derivatives by single precipitation with glycine or PEG. Possible improvements in stability and solubility of these products by use of additives.

Purification of factor VIII from SIP, SIRV and cryoprecipitated derivatives by two stage precipitations with glycine or PEG or both. Possible improvements in stability and solubility of these products by use of additives.

Behaviour of factor VIII on heparin-Sepharose and dextran sulphate-Sepharose.

13-281

## Development of factor VIII concentrates at PFL

### 1. Disadvantages of existing English factor VIII concentrates

The intermediate purity factor VIII concentrate (8IP) produced at PFL and BPL is not as potent or as easily soluble as several commercial products. By reducing the volume of buffer used to extract cryoprecipitate the potency of the preparation can be increased to 10-15 iu/ml at the expense of a longer solution time, 15-45 min. The purity and yield are not diminished by this variation (8IRV), but it is difficult to deliver 250 iu in any of the large vials readily obtainable in the UK and self-administration of this most usual dose would require the solution of more than one vial. It is also doubtful whether existing plant at the two fractionation centres could convert the required volume of plasma to 8IP concentrate, primarily because of the volume to be freeze dried. This gives additional impetus to the development of a more potent and more soluble concentrate.

### 2. Minimum specification for self-administration

Doses for self-administration should meet the specification: 250 iu per vial, soluble in not more than 10 min. at room temperature to a peteney not less than 15 iu/ml. High specific activity is primarily important in the interest of solubility, but a reduction of e.g. fibrinegen and blood group antibody concentrations would be particularly welcome.

In the light of recent estimates of UK requirements, the yield of any new product to replace intermediate purity factor VIII nationally should be not less than 200 iu/kg plasma, whereas 150 iu/kg might be acceptable in a product initially for limited distribution.

### 3. Possible approaches to improved concentrates

The minimum specification might be met by a combination of small improvements in factor VIII recovery, e.g. by increasing the mean factor VIII activity of the starting plasma, improving the ratio of factor VIII to protein in the extracted cryoprecipitate, reducing processing losses or improving the solubility of the existing 8IRV product by the use of alternative salts, excipients or other additives; indeed, the PFC product corresponding to 8IRV is very near the minimum specification. Some of these approaches should be pursued even if they do not immediately lead to an ideal product, because they may result in a better yield of any concentrate, however prepared. In particular, improved liaison with Oxford and other RTCs provides immediate opportunities to assess the cost-effectiveness of improvements in plasma procurement.

It is rather more likely that, to achieve the suggested minimum specification reliably from "average quality" plasma, further processing of the intermediate purity extract will be required. Earlier disappointing results from the large scale application of "high purity" methods might be improved by using protease inhibitors and other "stabilisers" such as heparin. There has been no systematic study of the relative solubilities of factor VIII and fibrinogen in a medium corresponding to 8IP or 8IRV extract, under varying conditions of pH, temperature and glycine or PEG concentrations. More selective extraction of cryoprecipitate has also been neglected. A rapid survey at PFL of several of these variables has already yielded results meriting more detailed study, e.g. the possibility of improving the specific activity of 8IP or 8IRV by 20-30% with little loss of yield.

13-282

Some workers believe that factor VIII activity may best be preserved by avoiding precipitations altogether, and there are already a number of methods based on solid-phase adsorbents, including affinity reagents such as heparin - Sepharose. The rate at which PFL pursues such long term possibilities would vary inversely with the success promised or achieved by more conventional tactics.

4. Means required

It is essential that this work be carried out where promising variants can be tested early on a realistic production scale and where good facilities exist for clinical testing. PFL is well placed in these respects and has an unrivalled organisation for the reliable assay of factor VIII in the many samples arising from the manipulation of several processing variables. At the moment, however, no member of staff with the appropriate talents can be spared from routine production or control duties to pursue the stated aims continuously and purposefully.

The allocation of one junior technician establishment, for a period of perhaps two years, would release a more experienced technician to work as research assistant to Dr. Smith and would permit the examination of many potential improvements of factor VIII concentrates. Given that there will be other limitations on the rate of progress (e.g. freeze drying or assay delays) this establishment might leave one or two man-days per week free for attention to other interesting concentrates, such as factor XIII, antithrombin III or improved factor IX. No experienced fractionator would guarantee that this work would lead to a conceptually new national factor VIII concentrate, superior in every way to existing ones, but even undramatic results recorded soberly and carefully may forestall more costly errors of investment in plasma procurement and fractionation plant.

13-283