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A HIGH-YIELD FACTOR VIII CONCENTRATE SUITABLE FOR ADVANCED HEAT TREATMENT R.V.McIntosh,N.Docherty,D.Fleming and P.R.Foster Protein Fractionation Centre, Scottish National Blood Transfusion Service, Edinburgh, EH17 7QT, U.K.

The heat treatment of Factor VIII concentrates in the freeze-dried state has been widely adopted to inactivate Human Immunodeficiency Virus (HIV) which may be present. However, the degree of heating that can be applied has been limited by adverse effects on product yield and quality (eg.solubility).The established SNBTS product (1) can be heated for up to 2 hours at 68°C in the absence of stabiliser and for up to 24 hours at 68°C in the presence of 2% sucrose.These conditions are believed to inactivate HIV but are not considered sufficient to destroy the hepatitis viruses.Therefore, a new product has been developed which can be heated more severely (eg.80°C for 72 hours) with the objective of destroying all potential viral contaminants while retaining good product solubility and a yield consistent with the maintenance of national self-sufficiency.The process combines Zn++ precipitation of cryoprecipitate (2) and Ca++ stabilisation of VIII:C (2,3) with developments in extraction, adsorption, formulation, freezing and freeze drying.

Cryoprecipitate is extracted in an equal volume of tris buffer; further purification is achieved by precipitation with cold zinc acetate and adsorption with Al(OH)3.The supernatant solution is stabilised by adding Na3 citrate, CaCl2, NaCl and sucrose; then concentrated and diafiltered to a final formulation of 20mM tris, 130mM NaCl, 30mM Na3 citrate, 4mM CaCl2 and 3% sucrose.The product is dispensed at 15ml (VIII:C 20iu/ml) for reconstitution in 20ml.A critical part of the process is a special 2-stage freezing procedure where the product is supercooled to -5°C before freezing to -50°C. Sublimation is completed with the product temperature held below -30°C at a pressure of 0.08 - 0.10mbar.

This process has been carried out at full-scale (750 litres plasma) giving a readily soluble, improved, intermediate - purity product with an VIII:C yield of 300iu/litre plasma after heat treatment.

1. P. R. Foster et al. Vox Sang. 42: 180-187, 1982
2. P. R. Foster et al. Thromb. Haem. 50: 117, 1983
3. P. R. Foster et al. Scand J Haematol. S40, 33: 103-110, 1984

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DEVELOPMENT OF A COAGULATION FACTOR X CONCENTRATE AS A BY-PRODUCT OF COAGULATION FACTOR IX PRODUCTION. Shirley I. Miekka, David B. Clark and Doris Menache, American Red Cross Biomedical Research and Development Laboratory, Rockville, MD, 20855, U.S.A.

The American Red Cross is developing a Coagulation Factor X (FX) concentrate to provide a safer alternative for replacement therapy in Factor X deficient patients, who can experience thromboembolic complications with current treatments. Based on a survey of hemophilia treatment centers, we estimate the frequency of the homozygous disorder to be approximately 1/150th that of hemophilia A, or about 65 patients in the USA. We have devised a method for producing FX as a by-product of our Coagulation Factor IX concentrate (FIX). The method starts with adsorption of cryoprecipitate supernatant plasma with DEAE-Sephadex resin followed by elution of Vitamin K-dependent coagulation factors. This material is adsorbed to sulfated dextran resin and Factors II and X are eluted by increasing the salt concentration. At 0.45 M NaCl, FII elutes quickly while FX is retarded and can be recovered essentially free of FII by collecting the slower eluting material. FIX is then recovered at still higher ionic strength. The pooled FX is concentrated, diafiltered and treated to inactivate viruses. Approximately 30% of plasma FX was recovered in pilot scale experiments (600 liters plasma). Specific activity was $>$ 50 FX units / mg protein corresponding to a purity of around 50% and 3000-fold purification over plasma. The ratios of Factors X : II : IX : Protein C were 1.0 : $<$0.03 : $<$0.03 : 0.2. The major contaminant, comprising nearly 50% of the protein, was found to be inter-alpha trypsin inhibitor (IaI), a serine protease inhibitor whose function in plasma has not yet been determined. This inhibitor is also present in the DEAE-Sephadex eluate and in the FIX concentrate. However, Western blot and HPLC analyses have shown that IaI is present in two different forms. In FX it behaves as expected for the IaI monomer (Mr = 160 kDa), while in the DEAE-eluate and in FIX it exists also in a higher molecular weight form ($\geq$400 kDa) corresponding either to aggregates, complexes or larger native species not previously described. The nature of the possible interaction of IaI with these coagulation factors is unknown and is currently being evaluated.

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RELATIVE THERAPEUTIC EFFICACY AND MOLECULAR WEIGHT DISTRIBUTION OF DRY-HEATED AND *n*-HEPTANE-HEATED PREPARATIONS OF FACTOR VIII. S.B. Abramson (1), J. Yang (1), E.D. Gomperts (2), C.K. Kasper (3) and E.J. Fedor (1). Alpha Therapeutic Corp., Los Angeles, CA, U.S.A. (1); Childrens Hospital, Los Angeles, CA, U.S.A. (2); and Orthopaedic Hospital, Los Angeles, CA, U.S.A. (3).

Recent reports by Kernoff *et al.* (1985) and Gomperts *et al.* (1987; a multicentered multinational trial) showed that Factor VIII (Antihemophilic Factor, or AHF) "wet" heat-treated in *n*-heptane (Profilate Heat-Treated®) presented a lower risk of transmitting non-A, non-B hepatitis and HIV than AHF products heated as lyophilized powders. No direct comparison has been reported, however, of the therapeutic efficacy of these products. We compared recovery and half-life *in vivo* for Profilate Heat-Treated® with those of the dry-heated products HT Profilate® and Koate HT®. Two sets of six subjects with severe hemophilia A were infused with either Profilate Heat-Treated® or a dry-heated AHF in a crossover trial, and blood samples were drawn at times from 10 min to 24 hr. Half-life was determined from a linear regression plot of log (plasma AHF) *vs.* time from 1 hr to 24 hr. The table gives the mean ± one standard deviation of initial recovery and half-life for each product comparison. Unpaired *t*-tests showed no significant differences between products. Spearman's rank analysis showed a high degree of correlation for both the initial recovery and half-life of each product pair.

Product	Recovery (%)	Half-life (hr)
Profilate Heat-Treated® ("wet")	108 ± 30	12 ± 3.3
HT Profilate® ("dry")	100 ± 38	11 ± 2.0
Profilate Heat-Treated® ("wet")	110 ± 16	13 ± 3.4
Koate HT® ("dry")	120 ± 29	13 ± 2.2

Analysis of molecular weight (MW) distributions of Factor VIII:C polypeptides in several commercial products using the method of Weinstein showed the majority of AHF in all products tested to have MW = 100,000 - 110,000. H.T. Factorate®, which exhibited a substantial amount of the AHF polypeptide whose MW approximates 210,000, is reported by the manufacturer to have a half-life = 11 ± 3.9 hr. We thus conclude that the 210,000 MW form of AHF is not required for therapeutic efficacy.

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PRODUCTION OF A HIGH-PURITY FACTOR VIII CONCENTRATE BY MEANS OF POROUS SILICA. D.W. In der Maur, P.J. Hoek, M.P.J. Piët, E. de Jonge, K. Toet and J. Over. Dept. of Development and Quality Assurance, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

Adsorption of proteins contaminating Factor VIII in cryoprecipitate to porous silica has been evaluated as a means to produce a high-purity Factor VIII concentrate for clinical use. At first, optimal conditions for the adsorption step have been assessed. A pore size of the silica of 1000 A, a pH of 6.6 and a contact time of 30 minutes gave the best results, while ionic strength and temperature were of no importance. The specific activity of Factor VIII in the effluent was directly proportional to the ratio of silica over cryoprecipitate solution, but the Factor VIII recovery was inversely proportional. At ratio's of about 2:1 the recovery was about 90% with a specific activity of 1.5 - 2.0 IU/mg protein.

Several techniques to concentrate Factor VIII from the effluent were evaluated. Of those tried (precipitation by ammonium sulphate, polyethyleneglycol or a combination of NaCl and glycin, freezedrying, and ultrafiltration using several types of membranes) ultrafiltration using hollow-fiber cartridges meant for kidney dialysis proved to be optimal: when stabilized by a mixture of amino acids the Factor VIII solution could be concentrated five-fold within 60 minutes at a 80 - 95% yield of Factor VIII with some additional purification. The stabilizers also allowed the final preparation to be heat-treated in lyophilized state for 72 hours at 60 °C or 30 hours at 70 °C.

The process scheme (cryoprecipitation, porous silica adsorption, ultrafiltration, sterile filtration, freezedrying and heat treatment) has been tried out 8 times at 200 - 300 liter plasma scale and showed highly consistent results. On average, the specific activity was 1.8 IU/mg, the Factor VIII recovery 56% relative to the Factor VIII content of the starting cryoprecipitate, and the Factor VIII concentration 28 IU/ml. The process is now being scaled up to 1,000 - 1,500 liter plasma, after which clinical evaluation will follow. It is anticipated that at full production scale the porous silica method combines good purifying capacity with a relatively high yield of Factor VIII.