

Notes of the XVII Congress of the World Federation of Hemophilia
Milan, 8th-13th June, 1986.

Report by J.K. Smith

Effects of virus inactivation procedures - clinical reports

- (309 p233). Suggested that human parvovirus (B19) could be transmitted by at least some heated concentrates, including Kryobulin TIM 3, Prothromplex TIM 4 and Provertin TIM 4, Koate HT, Preconativ and Kryobulin TIM 1. Although the virus seems unlikely to be a major source of morbidity in haemophiliacs, the observation might be interesting as an example of a tough blood-borne virus which resists several kinds of heat treatment.
- (318 p215). Described the largely non-symptomatic occurrence of NANBH, all within 12 weeks of infusion, in patients receiving Alpha factor VIII (3/8) and Hemofil (4/6).
- (317 p216). Reported another study of Hemofil T. 4/6 previously untreated patients got NANBH, but neither HBV nor HTLV III was transmitted.
- (155 p216). Found no virus transmission in six patients who had received Behringwerke factor IX concentrate, heated in solution, presumably the product described at Stockholm.
- (134 p233). Virtually repeated Kernoff's earlier publication on the "reduced" incidence of NANBH following the use of Alpha Profilate heated in n-heptane after freeze-drying. Among new points, he proposed the restriction of "previously untreated" status to patients who had had fewer than 60 units of cryoprecipitate, i.e. an estimated 1/20 risk of having encountered NANBH. The final incidence was 5/18 patients, or 2/9 batches. It was pointed out from the audience that 5/18 did not differ significantly from the incidence reported from Hemofil T. I think we should be more interested in the number of batches transmitting to at least two patients.
- (105 p243). Reported another study of Profilate and Profilnine, mainly carried out in the US. The protocol had called for LFTs at only 0, 2, 6, 12 and 26 weeks. Even then 7/31 patients had had to be excluded from the trial on grounds of non-compliance. 15 lots were used. 5/19 patients had significant ALT rises, of which one might be attributed to CMV and two were very late, >4 weeks and 52 weeks respectively. Only one of the cases could be attributed with any certainty to NANBH, and no patient was clinically ill or jaundiced at any time. I think this and other reports underline the need for LFT screening, at regular intervals, of a control group not treated with plasma protein concentrates.
- (167 p245). Summarised a French study of Koate HT, heated 72h at 68°. We were reminded that freeze-drying alone killed two logs of HTLV III, and the heat treatment at least another four logs. Selection of patients was virtually according to ISTH recommendations but follow-up, was only monthly. Of nine patients considered to have been more or less in compliance, two using the same batch had had significant ALT rises, tentatively attributed to NANBH. Neither had a clinical illness and two others using the same batch had had no LFT rises. Non-transmission of HTLV III was confirmed.
- (297 p244). Reported on the first trials of "steam heated" Kryobulin TIM 3 (details of heat treatment reported in another section). Full compliance with ISTH recommendations was asserted, but no chart was shown of the pattern of LFT testing actually achieved. Of 23 patients receiving seven lots, none had had evidence of NANBH or HTLV III, but

...of the ten non-vaccinated patients had developed markers for hepatitis B at eight, 14 and 26 weeks respectively, all from the same lot. Brian Colvin pointed out that the development of HB may have masked the presence of NANBH. Whereas the authors roundly attributed the LFT rises to HB transmission, Eibl was given a tedious ten minutes to explain why he thought that the patients had acquired their HB adventitiously, through living in filthy Southern Italy. This did not go down too well with his hosts, and does not explain why all three contracted HB after the same lot.

(223 p244). Was the only really satisfying trial, from any point of view. Trials of Behringwerke's Hemate P, pasteurised 60° 10h in solution under protection of sucrose and glycine, had hitherto been strongly suggestive of complete virus inactivation, but inconclusive because of faults in study design. The present study had followed ISTH rules, and provided evidence of good compliance on LFT testing. Of 21 patients who had gone more than four months, using 32 batches from US plasma unscreened for ALT, no patient had had an ALT rise greater than 2.5 times normal, and only one had shown ALT and AST at the upper limits of normal. Behringwerke are still very cagey about the effective yield, and I suppose that their use of US plasma suggests that national self-sufficiency is not their primary target.

Off the record, Colombo suggested a reason for Kabi having pulled out of the Melbourne meeting. Preconativ factor IX was definitely transmitting HTLV III, and probably NANBH and HB.

Effects of virus inactivation procedures, without clinical trials

(18 p214). Described dry heating of the Groningen two-precipitation heparin factor VIII at 68° for 48h with sorbitol or sucrose (2%).

(303 p215). Used some new fangled pharmacokinetic treatments to compare disappearance times for Kryobulin TIM 3, Koate HT and Hemofil T. All parameters were similar for the three concentrates and similar to previous experience with the unheated versions which could no longer be ethically studied.

(91 p217). Gave details of the steam treatments used by Immuno. Factor VIII is heated for 10h at 60° at 1190 ± 30 mBar. Plasminogen, IgG, C1 esterase inhibitor are treated similarly to factor VIII. Factor VII, factors IX, II and X, and FEIBA are heated for 10 hours at 60° at 1189 ± 7 mBar, then for an additional 1h at 80°, 1358 ± 30 mBar. Fibrin sealant is dry heated for 30h at 60°. It was not clear whether they had not yet got round to confirming steam treatment, or whether the components of the sealant did not survive well.

(11 p219). Found that the half-disappearance times of Immuno Kryobulin TIM 3, Behringwerke Hemate HS and an experimental preparation from Biotest were longer than found in previous studies. Interestingly, the FVIII:C half-life was much longer than that of FVIII vWF.

(146 p232). Was a difficult presentation of comparison of two Japanese concentrates with Immuno, Behringwerke, Cutter and Hyland prothrombin complex concentrates. All concentrates had <3 u/ml factor VII content, suggesting a change in some concentrates. All had coagulant activities less than 50% of the IX antigen. The Behringwerke concentrate gave a 50 kD electrophoretic band as well as the usual 60 kD band and was rather heterogeneous. They speculated that this might be IXa. There were no significant amounts of FEIBA, VIII:C or VIII vWF in any of the concentrates.

(71 p232). Is the first publication of the NRC work on dry heating freeze-dried cryoprecipitate. The final conditions under which the dried cryo was heated were: moisture <5%, vacuum <0.01 torr, heating 60° 72h. Sorbitol and mannitol had left poorly soluble products, as had glycine and tyrosine. Histidine, lysine, leucine, glutamine and arginine had caused discolouration. The best protection was with Synthamine mixture, with glycine and citrate at pH 6.4-6.5. 20 ml of diluent was used per 5 ml donation. FVIII:C suffered a 5-10% loss. Glucose was present at about 0.4 g/L, C-3 breakdown products were present and C- activation was doubled from 30-60 ug/ml. In vivo recovery was 80 ± 21% at 25 iu/kg, with a t_{1/2} of 16.7h. In seven infusions into vWF patients, 82 ± 25% was recovered, with the usual uncertainties over efficacy in stopping bleeding.

(224 p231) claiming to describe a new Behringwerke high purity concentrate FVIII C HS was withdrawn without explanation.

(122 p231). In a comparison between cryoprecipitate and Behringwerke pasteurised concentrate in vWD, the concentrate was deficient in HMW multimers, but appeared to normalise bleeding time and platelet retention more rapidly than cryoprecipitate. The heated concentrate did not give a secondary rise in FVIII:C. The ability of the concentrates to increase low and intermediate MW multimers seemed to be sufficient to give the desired haemostatic effect. It was not clear to me during this presentation whether the subjects were bleeding at the time or simply brought in for laboratory infusions.

(199 p233). Used an 8CRV/HL-like IP concentrate, heated 96h at 68° with 12.3% loss of FVIII:C over 40 batches. In vivo recovery was >90% and t_{1/2} over 6-72h was 15-20h. Reminiscent of HL and 8CRV, HMW multimers were absent from the unheated material but substantial polymerisation was found after heating (in fact all bands were found to be slower after heating). The heated concentrate showed greater functional activity in a platelet-adhesion test than the unheated version (approximately two-fold).

Use of FEIBA etc. in treatment of patients with factor VIII inhibitors

(261 p86). Described a fairly conventional use of Autoplex.

(253 p85). Described effective clinical results of using Hyate-C porcine factor VIII in 10 patients with inhibitors. The side effects were considered to be minor and anti-porcine antibodies were at "acceptable" levels.

(250 p210). Repeated Wensley's presentation at ISBT on the use of porcine factor VIII to treat haemophilia A patients without inhibitors. Treatment had been reasonably effective, but the rapid development of antibodies in 4-7 days made it unsuitable for long-term treatment, or of patients who might re-bleed. In private conversation, Wensley admits that this would no longer be the treatment of choice. The work was carried out several years ago when no virus-inactivated concentrate was readily available.

(22 p103). Discussed the experimental clinical use of a factor VIII preparation containing only FVIII:C light chain which lacks all FVIII:C coagulant activity but has been found to be the part of the molecule which usually binds to inhibitor antibodies. It was not clear whether Nordisk made this preparation from cryo-supernatant or, as seemed more probable from a later poster (33 p134), from a higher yielding initial capture from whole plasma, e.g. Cohn fraction I. Their interpretation

of the laboratory data, particularly on half-life, was extremely dubious and their speculations outran their evidence by miles. This work is reminiscent of Alan Johnson's idea from 15 years ago of preparing factor VIII from outdated plasma for mopping up patients' inhibitors before hitting them with active factor VIII concentrate. That project was unsuccessful too.

(187 p103). Described interim results from Trevor Barrowcliffe's infusions of FVIII-phospholipid into haemophilic dogs. Interpretation of results had been greatly confused by heparin release during anaphylactic reactions attributed to the factor VIII concentrate used, and a consequent disturbance of FVIII:C assays which needed the presence of protamine sulphate. The VIII-PL preparation had seemed to be less reactive and PL alone never gave anaphylaxis. FVIII:C recovery and half-life seemed to be similar with or without PL given at about the same time. It was claimed that the VIII-PL complex, in at least one of the dogs, had given a large increase in the recovery of FVIII:C in a haemophilic dog and an even larger increase in FVIII vWF recovery. His thesis still seems to be unproven.

(368 p102). Carried on the saga of the nature of FEIBA activity. Giles reminded us that, using the cuticle bleeding time (CBT) in haemophilic dogs, certain molar combinations of factor X and phospholipid (PCPS) appeared to bypass factor VIII. He showed that tissue plasminogen activator was also increased after infusions, and Protein C levels were diminished. He showed evidence that the consequent anticoagulant and fibrinolytic activities were counterbalanced by the coagulant effect of factor Xa and platelet phospholipid. Given the precise molar relationships required to have a beneficial effect, it seems unlikely that these observations will be exploited for regular human use.

Analytical methods

(294 p96) and (183 p96). Described two noteworthy new analyses of multimeric structure in vWD, both employing enzyme-linked antibodies rather than radio-iodinated antibodies.

(16 p102). Described a very direct method for measurement of FVIII antibodies, which depended on a very potent monoclonal antibody to VIII:C (Hybertech, Australia) coated on a tube. This in turn was coated with FVIII:C in the form of a concentrate. When this was incubated with a test plasma followed by a peroxidase-linked anti-human IgG, human antibody was picked up on the linking FVIII:C. This technique must have been tried and found wanting by many previous workers, presumably using less active antibodies on the tube.

(161 p131). Described the Royal Free/Speywood/Diagen depletion of normal human plasma to give a one-stage substrate. Cryosupernatant containing >1.2 g/L fibrinogen was adsorbed successively in-line with one anti-vWF monoclonal antibody, and two anti-VIII:C monoclonals, all linked to Sepharose by CNBr. FVIII:C was always reduced to <2% of normal and all other factors were >100% of plasma values, presumably after a degree of concentration. Successful field comparisons of the synthetic substrate against deficient plasma were reported. Privately, Diagen is obtaining the human plasma source from abroad, and can supply only the UK market. The US market will be supplied by a subsidiary of Porton Products in California.

AIDS-related issues

(107 p189). Documented a progression of disease in 17 patients who had become seropositive for HTLV III/LAV. Significantly fewer patients had been found to have viraemia one year after seroconversion. Only one of 19 patients had shown any clinical progression towards AIDS, but 13 out of 19 showed progressive falls in T4 and thrombocytopaenia.

(125 p189) was notable for pointing out a low incidence of seroconversion from Australian factor IX as compared with the corresponding factor VIII concentrate. Only one of 16 partners of seropositive males had seroconverted. The largest group of seropositive patients had no other sign of AIDS-related disease than high IgG levels which might be considered almost diagnostic.

(237 p190). Described the progress towards AIDS-related syndromes of 187 patients known to be positive in late 1985. The many details defy summarising, but suggest to me that haemophiliacs are going to suffer the same rate of progress towards AIDS as other risk groups, just a little later.

(51 p190). Confirmed no seroconversion in 35 patients treated with factor IX concentrates. They suggested that damage from concentrates probably started in 1978 and that progress from seropositivity to AIDS took a median 36 months (compatible with the last entry).

(295 p191). Described an unusual case of fulminant Kaposi's sarcoma, almost certainly due to treatment with commercial factor VIII and factor IX concentrates, in which no antibody was ever detected. Although total lymphocyte counts were said to be normal, it seems likely that in this case he had ceased to be able to produce antibody in the few months before death.

In a session on "Risk of transmission of LAV/HTLV III infection" (27 p138), (125 p139), (272 p140), (221 p140) found a very low risk of HTLV III transmission to spouses and close contacts, whereas an Argentinian group (111 p139) found a 24% incidence of seropositivity in sexual partners of seropositive haemophiliacs.

Miscellaneous side effects of concentrates

(203 p85). Reported evidence of HB infection, by the same batch of factor VIII concentrate, in two boys who had previously been assumed to be immune, having had evidence of anti-HBs on a previous occasion.

(127 p82). Reported a decrease in prekallikrein and an increase in serum levels of C1-esterase inhibitor at about 24 hours after infusion of Behringwerke Himate at 50 iu/kg.

Monoclonal antibodies to factor VIII

(161 p131). Has already described the use of monoclonals to prepare FVIII deficient plasma.

(312 p212). Summarised the properties of four anti-VIII:C monoclonals from an unspecified number of clones at 0.5 mg IgG per ml gel (CNBr-activated Sepharose 4B). 700 iu of a challenge of about 1500 iu was absorbed and 200-350 iu eluted by 50% ethylene glycol in pH 6 buffer. Other conversations suggested that pH was not important. This offers some hope of finding an anti-C with reasonable capacity and able to be eluted under reasonably mild conditions. Ethylene glycol is said to be relatively easy to remove by e.g. gel filtration or adsorption of the

VIII onto another matrix.

Factor VIII vWF

I was unable to attend the session reported from p235 of the abstract book.

(32 p112). Showed that it was the light chain (80 kD C-terminal end) which bound to FVIII vWF. They had a monoclonal antibody which reacted only with light chain and one which reacted with light chain or heavy chain depending on the presence metal ions.

Various tricks with factor VIII concentrates

(39 p113). Described the results of covalent bonding of PEG to factor VIII (Abuchowski ? JBC 252, 3578 (1977)), using PEG 5000 and cyanuric chloride. VIII:C diminished as the PEG content of the compound increased. The complex was less rapidly digested by plasmin in low concentrations and better protected against activated Protein C or low concentrations of thrombin (10^{-3} u/ml). It had not yet been tried even in animals.

(33 p134). Described the preparation of FVIII:C without the use of specific monoclonal antibodies described before. A "fibrinogen-rich precipitate" dissolved in EDTA buffer was adsorbed to phenyl-Sepharose eluted with glycol, adsorbed to CM-Sepharose and eluted with a sodium chloride gradient. The specific activity was 1500 iu/mg and the final VIII:C activity was less than 1% of normal. The yield of antigen was approximately 50% and the preparation blocked a natural factor VIII inhibitor without having any coagulant activity.

(337 p202). Gave some details of the production of Armour's "Monoclote". Cryoprecipitate was stabilised by adsorption with aluminium hydroxide, subjected to a Zimmerman anti-FVIII vWF insolubilised mouse monoclonal antibody and the eluate adsorbed to and eluted from aminohexyl-Sepharose. The product was finished and heated in the dry state, under unspecified conditions. Since their patent reports a 40% yield for the aminohexyl stage alone, it is difficult to believe their claim that the recovery is "commercially acceptable" or "as good as their previous high purity material". Before the addition of albumin stabiliser, the specific activity is 700-3500 iu/mg and there is no IgG present. They have found a normal recovery and half-life in haemophilic dogs and patients. There is a "low level of mouse protein" in the product which has not so far sensitised rabbits tested. Seven patients have gone to six months without detectable sensitisation to mouse protein. They are currently starting NANBH surveillance in previously untreated patients. They are conscious of the need to assay for DNA in the product. Some vWF is eluted with the VIII:C but is probably not functional.

(205 p217). Suggests that Behringwerke have now dropped a brief heat defibrination stage from the preparation of their pasteurised factor VIII concentrate. As already stated, the promised presentation of a new high purity concentrate from the same stable was withdrawn.

Hepatic dysfunction in haemophilia

(184 p83). Found that dysfunction originally found in 44% of 30 haemophiliacs had not progressed. The average AST level had fallen, platelet counts were unchanged, lymphocyte counts were only minimally diminished and only one patient had developed clinical thrombocytopenia.

(68 p147). Repeated the view of the Sheffield workers that biopsies were revealing an alarming level of progressive liver disease in haemophiliacs, related probably to NANBH. Clinical features of the disease occurred only very late, in the cirrhotic phase. LFTs do not distinguish chronic progressive and chronic active hepatitis or cirrhosis, which were again unrelated to factor VIII consumption. Increasing levels of serum IgG were indicative of increasing severity of cirrhosis or CAH. The patient's age was not a factor in progression to cirrhosis. Seroconversion to HTLV III did not itself increase IgG levels.

Immunodeficiencies in haemophiliacs

This topic will be reviewed more fully in a separate submission, but attention is drawn here to relevant abstracts.

(78 p104), (248 p104), (222 p104), (59 p170), (227 p171), (316 p171) (348 p 172), (102 p173), (53 p174), (273 p 174), (225 p174), (69 p176), (101 p194), (80 p218).

Biotechnology

(168 p112). Described safety tests in animals of Genentech/Cutter's recombinant factor VIII. Plasma and recombinant sourced factor VIII were labelled with I131 or I125, both losing about 50% of VIII:C activity on labelling. At a dosage of approximately 400 iu/kg, rabbits showed a similar T_{1/2} of about seven hours for radiolabel on either p or r factor VIII; the T_{1/2} for other activities including VIII:C were slightly shorter. In dogs, similar T_{1/2} for label was also found, but it was difficult to measure VIII:C against normal dog factor VIII levels. There was no adverse physical response in any case.

(327 p201). Extended this work to demonstrate that rVIII shortened the cuticle bleeding time (CBT) in haemophilic dogs. In crossover studies in which dogs were given alternately p and r FVIII, the two preparations could not be distinguished in immediate recovery or half-life at similar dosage. There were no unwanted side effects and it was concluded that effective vWF-C recombination occurred in vivo. One dog acquired an antibody to human factor VIII, but since both got p FVIII in the crossover study this would hardly be surprising. Both got anti-albumin from the carrier protein. Before addition of albumin, the product was said to have 5000 iu/mg purity.

These two presentations were held to justify proceeding to human clinical studies. No-one was volunteering how soon supplies of such material could be available for general use.

(14 p111). Suggested that the heavily glycosylated peptide 740-1649 of factor VIII, split off from the N-terminal and C-terminal end of the molecule by thrombin, is redundant for expression of FVIII:C activity. Whereas a mixture of gene products from the N-terminal and C-terminal pieces has no FVIII:C activity, cells transfected simultaneously with genes for both fragments produced a complex with substantial FVIII:C

activity. They concluded that the central 40% of the FVIII:C molecule is "not essential for activity nor for the assembly of an active complex".

(310 p113). Suggested that mRNA coding for factor IX was produced relatively late in the maturity cycle of hepatocytes, whereas factor X was expressed very early. All three chains of fibrinogen, AT III, and PC were expressed to approximately half adult normal levels by 12 weeks of growth.

(246 p201) from Brownlee's group in Oxford summarised the problems of expressing human factor IX in e.g. bacterial cells, and the conclusion that the post-translational requirements (12 carboxyglutamic acid residues, hydroxy aspartate at residue 64, aspartate linked carbohydrate at 157 and 162 in the activation peptide) made the choice of a mammalian cell more obvious. Although others had demonstrated higher concentrations than their 0.25 µg/ml of factor IX in culture medium, their products were either transient (without e.g. being transfected along with vaccinia) or not biologically active because metal binding sites were absent. He proposed that since the post-translational modifications might be rate limiting, extra copies of carboxylating enzymes should be cloned into the same cell, or that more efficient cells should be found. He discussed the possibility of incorporating the human factor IX gene not directly into a cell but into a retrovirus which would then infect cells. In this way human "normal" genetic material could be incorporated into deficient patients. For instance, IX-retrovirus have been made to infect mouse fibroblast, with expression of the virus, and more recently human lymphoblast cells had shown just detectable factor IX expression. Other possible hosts were rat embryo fibroblast and human skin fibroblast. At current efficiencies, the whole body mass would be required to raise factor IX concentration by 5%, so further improvements would be necessary before insertion of genetic material into the human gene could be contemplated as a potential "treatment" for genetic deficiencies.

Buffers added before Freeze drying

Na-phosphate, 12 H₂O - 46 g/l

Citric acid..1 H₂O - 5.5. g/l

Sucrose - 20 g/l

Trimethamol (Tris) - 4.6 g/l, pH adjusted to 6.9. (Osmolarity - 398 mosm)