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PROTEIN AGGREGATES IN HEATED BLOOD PRODUCTS. L. Freeman (1), V. Hornsey (2), D.S. Pepper (3), P.R. Foster (4), L. Winkelman (5) and J. Dawes (1). MRC/SNBTS Blood Components Assay Group, Edinburgh (1), Edinburgh and S.E. Scotland Blood Transfusion Centre (2), Scottish National Blood Transfusion Service Headquarters Laboratory, Edinburgh (3), Protein Fractionation Centre, Edinburgh (4) and Plasma Fractionation Laboratory, Oxford, UK (5).

Heating of blood products to reduce viral infectivity is now a standard practice. Such treatment may also modify the constituent proteins, reducing their activity or altering their structure with potentially harmful consequences for the recipient. Partially denatured proteins frequently form aggregates, which are often immunogenic and could precipitate immune complex formation, allergic reactions and kidney damage. In addition they may contribute to the development of AIDS after HIV infection by inducing a persistent state of T-cell activation.

Protein aggregate formation in factor VIII and factor IX (II + X) concentrates has been investigated by fast protein liquid chromatography (FPLC), which proved to be a rapid, convenient method for this purpose. Freeze-drying alone resulted in aggregate formation in intermediate purity FVIII concentrates, but not in FIX concentrates. However, aggregates were detected after heating the FIX concentrate at 80°C for 72h in the dry state. Dry heating of intermediate purity FVIII concentrates to 68°C for 24h also increased the content of protein aggregates, which contained fibrinogen and fibronectin but little IgG. In this product, the aggregate content after heating correlated with total protein concentration. A higher purity FVIII concentrate selectively depleted in fibrinogen and fibronectin also contained protein aggregates after freeze-drying, but heating this product at 80°C for 72h resulted in a relatively small increase in aggregate content.

Haemophiliacs receiving regular injections of heated concentrates are constantly exposed to protein aggregates. They should be monitored for any harmful effects, and manufacturers should aim to reduce the aggregate content of their products.

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CRYOPRECIPITATE COMPOSITION AS A FUNCTION OF PLASMA SOFTENING RATE. L. Winkelman and M. Pinnell. Plasma Fractionation Laboratory, Churchill Hospital, Oxford, UK.

Plasma for fractionation is commonly stored at <-30°. Some warming is necessary to soften the frozen plasma in order that it may be crushed prior to thawing to 0° for cryoprecipitation. In an investigation of softening conditions, small-scale experiments were performed using frozen plasma warmed to various temperatures (-15° to -2.5°) and held for varying lengths of time (0-40h). Cryoprecipitate was prepared and recoveries of fVIII:C, fibrinogen and fibronectin assessed. Yields of fVIII:C and fibronectin were independent of softening conditions, but the yield of fibrinogen was directly related to the temperature profile of the plasma. If held for periods >1h at >-10°, the weight of fibrinogen in the cryoprecipitate increased. In the conditions used in these experiments (which were chosen to reflect those which might be possible on a large scale), fibrinogen recovery ranged from 600-1700 mg/kg plasma. These findings have important implications for the preparation of fVIII concentrate from cryoprecipitate where subsequent purification may be significantly easier and final yields improved if the initial burden of fibrinogen is reduced.

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POSSIBLE ASSOCIATION OF HLA AND Gm WITH THE ALLOIMMUNE RESPONSE TO FVIII. H.M. Reisner (1), E.A. Reisner (2), D.D. Kostyu (2), B.C. Lubahn (1), C. McMillan (1), and G.C. White (1). Departments of Pathology, Medicine and Pediatrics, UNC-Chapel Hill and N.C. Memorial Hospital, Chapel Hill, NC, USA (1) and Departments of Pathology and Microbiology/Immunology, Duke University Medical Center, Durham, NC, USA (2).

Between 5 and 15% of individuals with severe hemophilia A are at risk of developing inhibitors (alloantibodies) to FVIII. Genetic factors are important in determining risk, but the nature of these factors is poorly defined. The human immune response to a wide variety of antigens has been associated with the HLA and/or Gm loci. Hence, we have investigated polymorphisms at these two loci in hemophilia A patients with and without inhibitors.

DATA SET: To date 127 hemophiliacs have been Gm or HLA typed. Forty-eight are inhibitor positive (I+) based on positive FVIII neutralization assays. This represents about 70% of all I+ hemophiliacs seen at UNC. To prevent familial bias, one member of each of 14 pairs of close relatives was randomly removed from the Data Set without regard to inhibitor status (Data Set 1). Twenty non-white individuals were also removed to constitute Data Set 2. GM TYPING: Samples were typed for Gm antigens 1, 3 and 5. Phenotype frequencies in Data Sets 1 and 2 did not deviate from expected values. I+ hemophiliacs showed an excess of Gm 1 in both Data Sets which was of possible significance (p = .13 and .21 respectively by chi square). Reanalysis of Data Set 2 to include only I- individuals without evidence of either circulating VIII:C or VIII:CAg (N=58) yields a p of .12. HLA TYPING: Analysis on 77 individuals in Data Set 2 (21 I+, 56 I-) has been done. In preliminary typing of HLA A, B, C, DR and DQ no significant differences in antigen frequency were found between the I+ and I- groups. INTERACTION BETWEEN HLA AND GM: A significant excess of Gm 1 I+ individuals was found among all HLA-A2 positive hemophiliacs (N=45, p=.034 by Fisher's exact test. This was not significant after correction for multiple comparisons). This suggestion of an interaction between HLA-A2 and Gm 1 in determining alloreactivity to FVIII will require further prospective evaluation for confirmation.

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TYPE III PRO-COLLAGEN PEPTIDE IN LIVER DISEASE IN HAEMOPHILIA R.S.Evely, F.E.Preston, D.R.Triger*, C.R.M. Hay, M.Greaves, J.C.E.Underwood**
University Department of Haematology, University Department of Medicine*, University Department of Pathology**, Royal Hallamshire Hospital, Sheffield, UK.

During the past 10 years we have carried out liver biopsies on haemophiliacs with biochemical evidence of chronic liver disease (CLD). To date 44 biopsies have been obtained from 35 patients. Histological diagnoses are Chronic Persistent Hepatitis (CPH) 24, Chronic Aggressive Hepatitis (CAH) 11 and Cirrhosis 9. Serial biopsies indicate that progressive liver disease is now a serious problem in haemophilia. Liver biopsy is not without risk and therefore it is important to identify factors which may be of value in predicting the nature of the liver disease or its progression. Since intra-hepatic fibrosis is a feature of CLD we measured Type III amino terminal propeptide of pro-collagen (PC III) by radio-immunoassay on samples taken within a mean of 4.8 months of the liver biopsy. A normal range was established as 4.3 - 15.7ng/ml on healthy subjects (median 7.0). Median values and ranges for patients with CPH (N=13), CAH (N=5) and cirrhosis (N=5) were 8 (5.4 - 23.4), 14.2 (7.2 - 19.8) and 14.2 (11.2 - 23.0)ng/ml respectively. Although pro-collagen III values tended to be higher in progressive liver disease (CAH and cirrhosis) this did not reach statistical significance. It would, therefore, appear that unlike serum IgG, pro-collagen III will not be a valuable predictor of progressive liver disease in haemophilia. A larger study is necessary to clarify this.