1260

THE ISOLATION OF COAGULATION FACTOR VIII FROM HUMAN BLOOD PLASMA BY AFFINITY CHROMATOGRAPHY. W. Riethorst (1), M.W.P.M. te Booy (1), T. Beugeling (1), A. Bantjes (1), J. Over (2) and W.G. van Aken (2). Dept. of Chemical Technology, University of Twente, Enschede (1) and Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam (2), The Netherlands.

The need for high quality concentrates of coagulation factor VIII (FVIII:C) for treatment of haemophilia A is increasing. As the purity of FVIII:C obtained with existing large scale methods is poor and yields are low, another method for the isolation of FVIII is being developed primarily to avoid losses incurred during cryoprecipitation.
Affinity gels were prepared by derivatizing Sepharose CL 4B with different positively charged ligand-spacer combinations. The adsorption of FVIII as well as the von Willebrand factor (VWF) from human blood plasma onto these gels was measured by a one-stage assay for FVIII:C, and enzyme immuno assays (ELISA) for FVIII:CAG and VWF:AG using monoclonal antibodies. The influences of pH, conductivity, ligand density, gel:plasma ratio, and length and composition of the spacer as well as the adsorption kinetics were studied to obtain information about the types of interactions responsible for bonding of FVIII to the gels. A combination of at least electrostatic and hydrophobic interactions was concluded to play a role in most cases.
At optimal conditions more than 90 % of FVIII could be adsorbed batch-wise from plasma at room temperature in less than one hour with a gel:plasma ratio of 1:20 (i.e. 2.5 g dried gel/1 plasma). In different runs 65-75 % of the FVIII:C applied was recovered by column-wise elution with a salt gradient. The eluate contained less than 0.34 % of the protein applied, which implies that FVIII was purified 190 times. Using fresh-frozen plasma (0.8 IU FVIII/ml) as a starting material for this one-step procedure the final specific activity was 2.3 IU/mg, which is significantly better than that obtained for FVIII isolated by cryoprecipitation. Furthermore, the F.VIII:C to VWF ratio in the eluate was approximately 1:1. The isolated FVIII:C was stable at room temperature and the supernatant plasma appears suitable for further fractionation. It is concluded that this method is worth scaling up and its use for purification of FVIII from other sources is anticipated.

1261

THE INFLUENCE OF L-LYSINE ON FACTOR VIII ELUTED FROM MATRIX BOUND DEXTRAN SULPHATE. P. Harrison, R.H. Saundry, G.F. Savidge. Division of Haematology, United Medical and Dental School, Guy's and St. Thomas' Hospital, Lambeth Palace Road, London, SE1. ENGLAND.

Factor VIII/vWF displays high affinity for matrix bound sulphate groups. Linear salt and pH gradient elution from Dextran Sulphate Sepharose at 4°C resulted in complete separation of the complex from major protein contaminants with typical yields of 70-90% vWF:Ag and vWF (RCoF). Elution in physiological calcium concentrations gave VIII and VIII:Ag yields of 35% and 65% respectively. Addition of L-Lysine (1.0M) to all buffers inhibited VIII/vWF binding, although lysine gradients (0-1.0M) gave comparable binding and elution profiles as 0-1.0M NaCl, but with impaired resolution between protein moieties. However, in the presence of L-Lysine, yields of VIII and VIII:Ag were significantly improved to 80% and 100% respectively when assay standardizations with appropriate lysine concentrations were performed. Furthermore, the binding of fibrinogen could be inhibited by 0.15M L-Lysine, 0.075M NaCl in the equilibrating buffer.

The presence of enzymic activity, as assessed by S-2222 and S-2238 in the eluting fractions could be abolished by the application of recrycprecipitated material pre-adsorbed with $Al(OH)_3$ and celite. Incorporation of lysine to the buffers with the associated increase in yields further supports the potential viability of the separation principle for large scale purification of Factor VIII.

1262

IMMUNOPURIFICATION OF FVIII/vWF FROM PLASMA

MEJAN O., DELEZAY M., FERT V., CHEBALLAH R., and BOURGOIS A. Immunotech, Luminy, Case 915, 13288 Marseille cedex 9, FRANCE

At present, antihemophilic factor concentrates available for hemophilia A therapy raise some problems :
- weak recovery of FVIIIC (20 to 25%)
- specific activity generaly lower than 5IU/mg
- loss of a large part of the von Willebrand factor, carrier of the FVIIIC
- necessity to inactivate viral contaminants by treatments which induce a decrease in FVIII recovery.

To solve these difficulties, an immunopurification process has been developed directly from plasma. A mouse monoclonal antibody (MAb) toward vWF, able to bind the FVIII/vWF complex under physiologic conditions and to release it in a suitable elution buffer preserving the FVIIIC activity has been selected. MAb coupling chemistry on gel beads has been optimised. The immunopurification process developed allows to treat 12 to 19 liters of plasma with one gram of antibody in each cycle (i.e. 6h.). Immunoadsorbant can be used during 10 cycles with standard quality plasma and with an average recovery of 50% for FVIIIC. This process warrants an important elimination of contaminant virus (10^4 for HBs) an a release of the mouse MAb in purified FVIII lower than 10^{-9}M. These properties could justify an industrial application of this immunopurification process to produce therapeutic hemophilia A fractions.

1263

LEWIS BLOOD TYPE HAS AN EFFECT ON THE PLASMA CONCENTRATION OF FACTOR VIII. K.H. Ørstavik (1), L. Kornstad (2) and H.M. Reisner (3). Institute of Medical Genetics, University of Oslo, Oslo, Norway (1), National Blood Group Reference Laboratory, Institute of Public Health, Oslo, Norway (2) and Department of Pathology, School of Medicine, University of North Carolina at Chapel Hill, NC, U.S.A. (3).

The plasma concentration of factor VIII is influenced by the ABO blood group. Individuals with blood group O have a lower concentration of both factor VIII coagulant activity, factor VIII coagulant antigen (VIIICAg) and factor VIII related antigen (VIIIRAg) than individuals with group A, B and AB. Thirty percent of the genetic variance of VIIIRAg concentration is due to the ABO blood group. The Lewis substances Le^a and Le^b are closely related to the A, B and H substances. We therefore examined the effect of the presence of these antigens on the plasma concentration of VIIICAg and VIIIRAg. The material was 74 monozygotic and 84 dizygotic twin pairs and 58 blood donors with ABO blood group O. VIIICAg was determined by a radioimmunoassay and VIIIRAg was determined by an electroimmunoassay. A higher mean concentration of VIIICAg (147%) and VIIIRAg (81%) was found in individuals with the Le^a antigen on their red cell surface compared to the mean concentration of VIIICAg (101%) and VIIIRAg (66%) in individuals who lacked this antigen. The difference was found in individuals with ABO blood group O only. Individuals with red cell Le^a antigen are non-secretors and individuals who lack this antigen but have the Le^b antigen are secretors of the A, B and H substances. The lowest factor VIII concentration was found in group O secretors. The effect of the Lewis phenotype on factor VIII concentration is therefore most probably due to an effect of the secretor locus. This finding may have practical implications for the diagnosis of carriers of hemophilia A. It has been shown that information on the ABO blood group improves the discrimination between carriers and normals. We found that the effect of ABO blood group on the total variance of VIIIRAg was higher in secretors (21%) than in non-secretors (8%). Since the frequency of secretors varies widely, it is possible that the importance of the ABO locus in carrier detection is different in different populations. Lewis blood typing in materials of carriers and normals are necessary to determine the effect of the Lewis phenotype in carrier detection.