DIFFERENT FORMS OF FACTOR VIII RELATED ANTIGENS IN ENDOTHELIAL CELL CULTURES, CRYOPRECIPITATE SUPERNATANT AND NORMAL PLASMA. I.R. Peake, A.L. Bloom and S.A.M. Shearn. University Hospital of Wales, Cardiff, U.K.

The nature of factor VIII related antigen (FVIIIIRAG) was studied in endothelial cell cultures (EC), cryoprecipitate supernatant (cryo-S) and gel-filtered plasma fractions (GPF). Antigenic reactivity (AR) was assessed using the Laurell electroimmunoassay (LA) and a two-site immunoradiometric assay (IRMA) using a non-coagulation inhibitory rabbit antiserum raised against a factor VIII concentrate prepared by cryoprecipitation and gel filtration. Electrophoretic mobility (EM) of FVIIIIRAG was assessed by two dimensional crossed immunoelectrophoresis (2DCIE). Ristocetin co-factor activity (RiCoF) was estimated using fixed platelets. In normal plasma the results of LA, RiCoF assay and IRMA were in agreement. FVIIIIRAG synthesised by EC showed increased EM and, in the IRMA decreased AR when compared to LA often with non-parallel dose response curves (DRC). This effect was not prevented by the presence of protease inhibitors in the culture medium. RiCoF activity was reduced compared with LA and was related to the parallelism of the DRC in the IRMA. In cryo-S and the late eluting GPFs increased EM of FVIIIIRAG was seen, and in these preparations also the DRC of the IRMA were not parallel to the normal plasma DRC. AR and RiCoF activity were disproportionately reduced when compared to the LA. It is concluded that the IRMA is sensitive to the presence of different forms of FVIIIIRAG, particularly those which exhibit low levels of RiCoF activity and increased EM. Some of these forms are present in normal plasma.

MICROHETEROGENEITY OF HUMAN FACTOR VIII. J.A. van Mourik, J.A. Hellings and E.M. Hoorweg. Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

Human factor VIII is a glycoprotein and tends to form a variety of large aggregates, the presence of which was demonstrated by a number of techniques including the use of large pore gel chromatography and electrophoresis using a polyacrylamide gel system designed for high molecular weight aggregates. Factor VIII aggregates can be fractionated in part according to molecular size. Reduction of these polymers by 2-mercaptoethanol results in presumably identical fragments suggesting that all polymers, though differing in size, are composed of identical subunits.

Factor VIII aggregates are particularly sensitive to proteolytic breakdown by trypsin and plasmin as judged by large pore polyacrylamide gel electrophoresis. Short-term incubation of factor VIII with trace quantities (equivalent to plasmin- or trypsin-like activities present in normal plasma) of these enzymes respectively results in substantial fragmentation with concurrent loss of both factor VIII procoagulant and Von Willebrand factor activity. However, factor VIII activity is lost prior to discernible protein fragmentation whereas Von Willebrand factor inactivation is associated with advanced protein degradation. When the ionic strength of the medium is lowered the susceptibility of factor VIII to proteolytic breakdown by trypsin is increased dramatically. Interestingly, degradation of factor VIII by plasmin is not affected by the ionic strength. These data, then, provide conclusive evidence of microheterogeneity of normal human factor VIII and may account for the observed heterogeneity of factor VIII on crossed immunoelectrophoresis.

STABILIZATION OF THE ANTIHEMOPHILIC FACTOR (VIII:AHF) BY THE VON WILLEBRAND FACTOR (VIII:VWF): A POSSIBLE MODEL FOR VON WILLEBRAND'S DISEASE. H.J. Weiss, I.I. Sussman and L.W. Hoyer. Columbia University, New York, New York, USA and Univ. Connecticut School of Medicine, Farmington, Conn., USA.

When compared with VIII:AHF in normal citrated plasmas, VIII:AHF activity showed increased lability at 37°C in the 'late' post-transfusion plasmas (VIII:AHF >> VIII:VWF) of a patient with von Willebrand's disease, but not in the 'early' post-transfusion plasmas in which VIII:AHF ~ VIII:VWF. VIII:AHF was also labile in the baseline plasmas of 3 patients with von Willebrand's disease in whom VIII:AHF >> VIII:VWF. In two of these patients the mobility of Factor VIII antigen (on crossed immunoelectrophoresis) was increased. (VIII:AHF was not excessively labile in 4 other patients in whom VIII:AHF ~ VIII:VWF). In all of the above cases, VIII:AHF was stabilized by the addition of either purified von Willebrand factor or plasmas of patients with hemophilia, but not by plasmas of patients with severe von Willebrand's disease. Thus, VIII:VWF may serve to stabilize VIII:AHF and this might explain the post-transfusion findings in von Willebrand's disease.