**PHYSICOCHEMICAL CHARACTERISTICS OF THE HUMAN FACTOR VIII-POD WILLEBRAND FACTOR OLGOMERS. P.A. Boshuis, A.G. Seijen and I.A. Achter. University of Amsterdam, Amsterdam, The Netherlands.**

Analytical ultracentrifugation of the factor VIII-Von Willebrand factor showed an apparently homogenous protein with an S20, w of 29. With the diffusion coefficient D of 1.24 x 10^-7 cm^2/sec. (obtained by double diffusion of the protein and the immunochemically equivalent amount of rabbit anti-factor VIII) a mean mol.wt. of 2.2 x 10^6 and a friction ratio of 1.9 are derived. Turbidity measurements confirmed the high mol.wt. and resulted in an estimation of the volume of the protein. The free electric mobility was found to be 3.6 x 10^-9 cm^2/Vsec at pH 5.80, while iso-electric focussing patterns of the native protein and the polypeptide (mol.wt. 260 000) obtained by reduction in the presence of urea exhibited a maximum at about pH 4.5. The apparent D found upon electrophoresis is an order higher than given above and is dependent on the field strength. The heterogeneity indicated by this phenomenon was confirmed by reverse electrophoresis (yielding a decrease in the apparent D). Since continuous immuno precipitation lines are found upon cross-electrophoresis, we interpret the results in terms of a series of closely related oligomers with a mean number of 8 polypeptide chains and a variation from about 6 to 10.

**THE FACTOR VIII MOLECULE IN ACQUIRED VON WILLEBRAND'S DISEASE. F. E. Preston, R. C. Nails and E. Sampson. University Department of Haematology, Royal Infirmary, Sheffield, England.**

Four patients with acquired von Willebrand's disease have been studied. The diagnosis in each case was based on acquired bleeding disorder, negative family history, prolonged bleeding time, low procoagulant factor VIII (F. VIIIc) and factor VIII related antigen (F. VIII:Ag) activity and impaired ristocetin-induced platelet aggregation responses.

Cell filtration studies were performed on plasma samples from each of the four patients and the fractions tested for F.VIIIic activity using a modified kaolin cephalin clotting time. Samples from each patient showed two peaks of procoagulant activity compared with one peak obtained on samples from controls, haemophiliacs and classical von Willebrand's syndrome.

When incubation mixtures of acquired von Willebrand plasma and a source of normal factor VIII are examined by similar gel filtration techniques, it can be shown that the normal F.VIIIc becomes dissociated into sub-units of varying size.

Similar results have been obtained by incubating mixtures of the patients' IgG obtained by an exchange chromatography and normal sources of factor VIII.

It is concluded that acquired von Willebrand's disease is probably due to an antibody directed against the factor VIII molecule.


The synthesis of coagulation factors, including procoagulant factor VIII (FVIIIc) and factor VIII related antigen (FVIII:Ag) was studied using the isolated perfused rat liver. The perfusion fluid consisted of rat blood cells suspended in Tyrodes solution containing bovine albumin. Synthetic function was confirmed by the addition of 15S 1,2-thrombin to the perfusion medium. Using two-dimensional crossed immunoelectrophoresis and autoradiography progressive incorporation of radioactivity into plasma protein was demonstrated. This was inhibited by cyclobenzaprine. Coagulation factor assay demonstrated the synthesis of factors II, IX and X and of factors V and VIIc. Demonstration of synthesis of FVIII:Ag was attempted using an immunoelectrophoretic assay and a cross-reacting rabbit anti-human antiserum. FV and FVIII:Ag synthesis was more clearly demonstrated when the perfusion fluid was depleted of leucocytes and platelets. Addition of Warfarin inhibited the synthesis of factors II, IX and X but not of factors V and VIIc. Cyclobenzaprine completely inhibited synthesis of all coagulation factors but actinomycin acted only after a latent period. No coagulation factor synthesis was demonstrated in the perfusion system without a liver. The results confirmed the functional capacity of the isolated perfused liver for synthesizing proteins and the vitamin K dependent factors and demonstrated similar kinetic features for the synthesis of factors V, VIIc and, possibly, FVIIIc.