MIXED POSTERS II

Coagulation

THE SEPARATION OF WILLEBRAND FACTOR FROM FACTOR VIII RELATED ANTIGEN. E.S. Barrow, H.M. Reisner and J.B. Graham. University of North Carolina School of Medicine, Chapel Hill, N.C., U.S.A.

Factor VIII (F.VIII) in normal plasma: a) shortens the prolonged clotting time of hemophilic plasma (F.VIII coagulant, VIII:C), b) precipitates with heterologous antisera (F.VIII related antigen, VIIIR:AG), and c) together with the antibiotic Ristocetin, aggregates platelets (F.VIII related Willebrand factor, VIIIR:WF). VIII:C has been shown by others to be separable from VIIIR:AG-WF proteins by gel filtration with buffers of high ionic strength. No one has, to our knowledge, clearly separated VIIIR:WF from VIIIR:AG. We seem to have accomplished this by sequential use of two antibodies to F.VIII. The IgG fractions of a precipitating rabbit anti-human VIII and of a human, non-precipitating anti-VIII were separately bound covalently to CNBr-activated Sepharose. The rabbit antibody had a high affinity for VIIIR:AG, but a low affinity for VIII:C and VIIIR:WF. Passage of human plasma over the rabbit antibody column completely removed VIIIR:AG, but not the VIII:C or VIIIR:WF. The VIIIR:AG-free plasma was then sent over the human antibody column which removed only VIII:C. The effluent retained 60% of the original VIIIR:WF activity but had no measurable VIII:C or VIIIR:AG. Bio-Gel A-15 filtration of the VIIIR:WF resulted in elution of the activity in the Vo fractions. These data suggest that the VIII:C, VIIIR:AG and VIIIR:WF activities may exist in plasma as separate entities.

SOME CHARACTERISTICS OF FIBRIN MONOMER PREPARATIONS MADE FROM DISSOLVED FIBRIN CLOTS OR FROM FIBRINOGEN AND IMMOBILIZED THROMBIN IN UREA. F. Brosstad, H.C. Godal and P. Kierulf. Haematological Research Laboratory, Department IX, Ullevål Hospital University Clinic, Oslo, Norway.

Fibrin monomer was prepared from purified human fibrinogen by the action of insolubilized thrombin in the presence of 3.3 M urea. As judged by N-terminal amino acid analyses, complete conversion of fibrinogen to fibrin was achieved. The preparation proved homogeneous by gel chromatography and SDS electrophoresis and had retained clotting abilities identical to those of fibrin monomers arising from fibrinogen exposed to large amounts of thrombin. No changes were observed after freezing in liquid nitrogen or after storage at +4^{o}C for 4 weeks. As to homogeneity and ability to polymerize the preparation proved superior to those fibrin monomer preparations obtained by dissolution of polymerized fibrin in various solvents.