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CHARACTERIZATION OF THE IN VITRO "FACTOR VIII BYPASSING ACTIVITY" D.L. Aronson and J. Bagley. Coagulation Branch, Division of Blood and Blood Products, Bureau of Biologics, Bethesda, MD, U.S.A.

The in vitro correction of the prolonged APTT of hemophilic plasma has been ascribed to an uncharacterized entity "Factor VIII Bypassing Activity." Such products also correct the prolonged APTT plasma deficient in Factor IX, Factor X and Factor XII, but not of Factor V deficient plasma. Correction of the APTT in Factor VIII deficient plasma by early stage coagulants such as Factor XII_a, Kallikrein and Factor IX_a is minimal. These results indicate that this in vitro activity acts at the level of either the activation of Factor X or the activation of prothrombin.

A coagulant has been prepared from serum by barium precipitation, heparin-agarose, DEAE cellulose and high pressure liquid chromatography (HPLC). The in vitro coagulant properties are similar to "activated" prothrombin complex (Autoplex) and the biologic and chemical properties are identical to activated Factor X.

Infusion of the partially purified serum coagulant into normal dogs was well tolerated and, in contrast to Factor IX concentrates, gave no signs of DIC. Infusion into bleeding hemophilic dogs had no hemostatic effect. It is concluded that a major portion of the in vitro potency of activated prothrombin concentrates is due to activated Factor X, a material which when infused has no in vivo hemostatic effect.

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CLEAVAGE OF REDUCED FACTOR VIII/VON WILLEBRAND FACTOR SUBUNITS BY THROMBIN OR FACTOR X_a. G. A. Vehar. Department of Biochemistry, University of Washington, Seattle, WA USA.

Purified Factor VIII/von Willebrand Factor (FVIII/vWF) was obtained from bovine plasma using ammonium sulfate and glycine precipitations, tricalcium citrate adsorption, and Sepharose CL-4B gel chromatography. The protein was concentrated with ammonium sulfate and dialyzed against 0.05 M Tris-HCl, pH 7.4, containing 0.15 M NaCl. Partial reduction of these preparations was obtained using a 2.5 hr incubation at 20°C with 5 mM dithioerythritol, followed by dialysis to remove the reductant. The addition of a 19 to 1 molar ratio (110 to 1 wt. ratio) of FVIII/vWF to thrombin resulted in a time dependent cleavage of the reduced preparation, as detected by SDS gel electrophoresis. A 50 min. digest resulted in the cleavage of the 220,000 dalton subunit to products of 145,000 and 140,000 daltons. Factor X_a, in the presence of calcium and phospholipid, also cleaved the reduced protein when present at a 14 to 1 molar ratio (75 to 1 wt. ratio) of FVIII/vWF to factor X_a. A 50 min. digest resulted in fragments of 200,000, 175,000, and 55,000 daltons. No cleavage occurred under these conditions if the native, unreduced FVIII/vWF was used. Factors XII_a, XI_a, IX_a and activated protein C did not cleave the reduced or unreduced preparations. The observed cleavages of FVIII/vWF by thrombin or factor X_a do not, however, correlate with the alteration of any known function of this protein complex.

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INTERACTION OF FACTOR VIII/VON WILLEBRAND FACTOR WITH PHOSPHOLIPID VESICLES. J.E. Brown and L.-O. Andersson. Department of Pathology, University of California, San Diego School of Medicine, USA, and Biochemistry Research Department, Kabi AB, Stockholm, Sweden.

The interaction of factor VIII/von Willebrand factor with phospholipid vesicles was studied using sucrose density gradient ultracentrifugation. Purified VIII/vWf (Kabi) sediments as a complex in the lower third of a 5-30% sucrose gradient centrifuged for 18 hours at 160,000 g. Addition of sonicated phosphatidylserine-phosphatidylethanolamine vesicles at concentrations above 250 µg/ml results in complete separation of VIII:C from vWf, the former appearing with the phospholipid in the top of the gradient and the latter sedimenting as before. At lower levels of phospholipid, vWf competes for binding of VIII:C. The separation at higher levels of phospholipid is obtained in the presence of DFP and Trasylol, does not require calcium and occurs with plasma as well as purified preparations. Activation (7-10-fold) of purified VIII/vWf by thrombin (10^{-3} units/ml) results in the formation of a slowly sedimenting component containing essentially all of the VIII:C activity with little or no associated vWf. The thrombin-activated VIII:C is strongly bound to phospholipid vesicles and is more stabile than uncomplexed VIII:C (thrombin-activated). By varying the concentration of thrombin activated VIII:C with a constant (5 µg/ml) concentration of phospholipid, a Scatchard binding plot was obtained and a dissociation constant of 2.5×10^{-9} M estimated (assuming a molecular weight for VIII:C of 90,000) for the thrombin-activated VIII:C-phospholipid complex. These studies suggest that thrombin activated VIII:C binds to exposed phospholipid on released platelets concentrating the factor X activator complex.

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THE DEVELOPMENT OF MONOCLONAL ANTIBODIES TO FACTOR VIII RELATED ANTIGEN (VIIIRAG). J.E. Thomas, R.A. Furlong, J.C. Giddings, I.R. Peake and A.L. Bloom. Department of Haematology, Welsh National School of Medicine, Cardiff CF4 4XW, U.K.

A human VIIIRAg rich fraction was prepared from human factor VIII concentrate by gel chromatography on Sepharose CL6B to give 40 u/ml VIIIRAg and 0.06 u/ml factor VIII clotting antigen (VIIICAg). Balb/c mice were immunised with this fraction (1-2 u VIIIRAg per mouse) and polyclonal antibody to VIIIRAg was detected in the serum of the mice by conventional immunodiffusion and by a specially developed immunoradiometric test (IRMT). This test utilised polystyrene tubes coated with polyclonal (sheep) antibody to VIIIRAg which were then incubated with a source of human VIIIRAg (pooled normal plasma). After incubation with mouse anti human VIIIRAg the bound mouse immunoglobulin was detected by binding of ^{125}I labelled rabbit anti mouse IgG. This was prepared from commercial rabbit anti mouse IgG by immunoadsorption and elution from immobilised mouse immunoglobulin. Using the IRMT a monoclonal antibody to VIIIRAg was detectable at a dilution of 5×10^5. Mice with the highest serum antibody titre were selected, hyperimmunised and used as spleen donors. Spleen cells were harvested, fused with homologous NS-1 myeloma cells and cultured in HAT selective medium. Hybrids producing specific antibody to VIIIRAg were detected by the IRMT and cloned by the limiting dilution method. Antibodies were tested for their activity against VIIIRAg and factor VIII related ristocetin cofactor activity, and also for any activity against VIIICAg and procoagulant factor VIII.