FACTOR IX RELATED ANTIGEN IN HEMOPHILIA B. H.C. Yang and P.H. Levine. The Memorial Hospital and the University of Massachusetts Medical School, Worcester, Massachusetts, U.S.A.

The presence of factor IX related antigen (FIXRA) was studied in 15 hemophilia B patients, 20 normals, 2 obligate carriers of hemophilia B, 10 hemophilia A patients and 2 patients on coumadin therapy. A monospecific rabbit antiserum for factor IX was used in a counterimmunoelectrophoresis (CIEP) system. Only 3 out of the 15 hemophilia B patients, representing 3 of 10 pedigrees, had a factor IX related antigen as demonstrated by a precipitin line in CIEP; the other 34 subjects all had demonstrable FIXRA. The presence of FIXRA in hemophilia B did not correlate with the factor IX procoagulant level. The hemophilia B group with FIXRA could partially neutralize a human inhibitor to factor IX. Hemophilia B is a heterogeneous disease in which a minority have a factor IX related antigen.

BOVINE FACTOR VIII. ANTIGENS AND BIOLOGICAL ACTIVITIES. G. Casillas, C. Simonetti and A. Pavlovsky. Instituto de Invest. Hematologicas. Buenos Aires. Argentina.

The procoagulant (PcgF) and the platelet aggregating (PAF) activities of bovine F. VIII were studied in order to establish their relation to the antigens A_1 and A_2, antigens similar to those synthetized by von Willebrand and hemophilia A patients respectively. Studies of stability to different agents (temperature. EDTA, thrombin, etc.) allowed us to establish that VIII (PcgF) and VIII (PAF) are not mutually dependent. An homologous antibody of low species specificity against human F. VIII binds specifically with VIII (A_1) antigenic moiety of bovine F. VIII. The complex so formed is purified by gel filtration and has VIII (PAF) activity but not VIII (PcgF) activity indicating that VIII (A_1) and VIII (PcgF) activities associate. Another complex, formed by bovine factor VIII and a rabbit antibody against the VIII (A_2) moiety, was prepared. This complex has VIII (PcgF) activity but not VIII (PAF) activity, indicating that VIII (A_2) and VIII (PAF) associate. The complex and its procoagulant activity sediment after centrifugation and may be recovered by suspension of the precipitate. The following scheme showing the relationship between the antigenic moieties and the activities of bovine factor VIII is proposed:
VIII (A_1-PcgF) (A_2-PAF).

THE LARGE SCALE PREPARATION OF CLOTTABLE FIBRINOGEN-FREE, HIGH PURITY, HIGH POTENCY FACTOR VIII CONCENTRATE. L.F. Fekete, W.L. Wilson, and R.L. Bick. Bay Area Hematology, Santa Monica, Ca. USA

Crude Factor VIII was initially extracted from plasma as cryoprecipitate. This crude concentrate was treated with high molecular weight polymer F-48 to remove the bulk of fibrinogen. The yield of Factor VIII at this point was 63% theoretical and 42% actual (the starting plasma contained .66 units/ml VIII). Total protein in the cryoprecipitate was 2.2 gm% and 0.93 gm% after removal of fibrinogen bulk. The next step was addition of a thrombin-like enzyme, at a concentration of 0.5 units, with the resultant removal of remaining fibrinogen. After clotting out residual fibrinogen, resultant fibrin strands were removed by high speed centrifugation. The final product gave a theoretical yield of 65% and an actual yield of 28%; thus, 65% of the initial Factor VIII was recovered in the final product. Prior to lyophilization, the final product was stabilized with albumin. After lyophilization, no loss of activity occurred and solubility was excellent. Protein electrophoretic analysis revealed 70% in the albumin region, 7% in the alpha & beta globulin region, and 20% in the gamma region. Tests for hemolysins were negative and isoagglutinin titers were 1:64. Plasminogen and plasmin were undetectable and fibrin(ogen) degradation products were 80 ug/ml. The large scale preparation of this fibrinogen-free Factor VIII concentrate may prove highly useful in sparing the hemophilic patient fibrin(ogen) deposits in the kidneys and other organs, a complication of existing concentrates. In addition, much higher potency in much less volume may be achieved with this material. The cost for large scale preparation of this material should be the same as for existing concentrates.