THE APPARENT SEPARATION OF FACTOR VIII RELATED ANTIGEN AND COAGULANT ACTIVITY FROM VON WILLEBRAND FACTOR ACTIVITY BY AN IMMUNOADSORBENT. H. A. Cooper, D. Lee, M. A. Lamb and R. H. Wagner. University of North Carolina, Chapel Hill, N. C., USA.

An antibody was raised in rabbits to the small active fragment of human factor VIII. This fragment was obtained by Ca^{2+} dissociation of a human factor VIII preparation made from a multidonor pool of plasma. After two adsorptions with 0.1 volume of normal human plasma, the antibody neutralized the F. VIII coagulant activity of normal human plasma, but did not precipitate with any plasma or plasma fractions nor did it neutralize vWF activity as measured by ristocetin aggregation of fixed washed platelets. A solid phase immunochemical reagent was prepared by CNBr binding of the partially purified rabbit antibody to 1% agarose beads. Non-immune beads were similarly prepared with IgG fractions from a normal non-immunized rabbit. Using a batch technique the beads were studied for their ability to remove F. VIII coagulant, F. VIII Ag, and vWF activity from normal human plasma. Assay of the supernatant plasma after 2 hrs, 22°, from 10 replicate experiments gave the following results for residual activity, as per cent of non-immune bead control: F. VIII (37.5±4), F. VIII-Ag (30.8±9.7), and vWF (72.1±16). The experiment was repeated with 6 replicate samples with higher ratio of beads to plasma with essentially similar results. This unexpected separation of F. VIII-Ag from vWF activity prompted further investigation into how these activities are related to the molecular structure of F. VIII and vWF.

ISOLATION OF SPECIFIC HOMOLOGOUS ANTIBODIES TO FACTOR VIII BY IMMUNOADSORPTION. J.-M. Lavergne, D. Meyer, J. Koutts, N. Ardaillou, J.P. Girma and M.J. Larrieu. Institut de Pathologie Cellulaire, Hôpital de Bicêtre, Paris, France.

Current immunological studies of Factor VIII use heterologous antibodies which predominantly measure Willebrand Factor (WF) and give little information on Factor VIII procoagulant activity (VIII:C). Purification of homologous antibodies specific for VIII:C has been hampered by the fact that they do not form immune precipitates. We have attempted to isolate such antibodies by solid phase immunoadsorption and subsequent elution. Human Factor VIII was specifically bound to goat anti-human Factor VIII IgG previously immobilized onto Sepharose 6 B beads. IgG isolated from a Haemophiliac with a high titer anti-VIII:C antibody (700 Oxford U/ml) was labeled with 125-I and reacted for 72 hours with these beads. The column was then washed with 0.1 M glycine, 0.5 M NaCl, pH 10 buffer to remove non specifically adsorbed material. Specifically adsorbed material was then eluted with 2.5 M $MgCl_2$, pH 7.3, and the peak of radioactivity was filtered on Biogel A-5 m. Both anti-VIII:C and radioactivity were recovered in three distinct peaks. The third peak, corresponding to IgG, contained anti-VIII:C activity with a 10 fold purification as estimated by specific radioactivity. The second peak, eluting just in front of the IgG, had half the specific radioactivity of the third peak. The first peak, corresponding to the Vo, contained Factor VIII related antigen and very little anti-VIII:C activity. The pattern by SDS-polyacrylamide gel electrophoresis is compatible with the existence of Factor VIII (WF-VIII:C)-anti-VIII:C complexes in the first peak; VIII:C-anti-VIII:C complexes in the second, and free anti-VIII:C IgG in the third one. Thus the method leads to the formation of stable VIII:C-anti-VIII:C complexes, allowing the purification of specific human anti-VIII:C antibodies.

THE ACTION OF IMMOBILIZED THROMBIN ON FACTOR VIII. J. E. Brown, C. Carton and C. Hougie. Department of Pathology, University of California, San Diego, La Jolla, California, U.S.A.

The utility of immobilized enzymes in blood coagulation studies is readily apparent. An enzymatic transformation can be stopped by centrifugation and removal of the enzyme beads. This could leave an "activated" factor free of the contaminating "activator". The further degradation which prolonged exposure to the enzyme often produces can thus easily be stopped. The action of immobilized thrombin on factor VIII warranted testing, since we have observed that hirudin added to a factor VIII solution that has been maximally activated with thrombin prevents the subsequent destruction of "activated" factor VIII. Thrombin was covalently bound to agarose and shown to be active in hydrolyzing a synthetic tripeptide (S-2160) with a pH optimum of 8.5. Tested on human or bovine plasma, immobilized thrombin had no effect on factor VIII activity over 24 hours. Incubated with purified bovine factor VIII (100 units/ml) and tested at various concentrations, no activation of factor VIII activity could be observed. Slow destruction of activity was noted, so that in 16 hours only 20% of factor VIII activity remained compared with glycine-agarose beads as control.

It was assumed that a complex of factor VIII and thrombin was necessary for the activated state. To test this, thrombin was labeled with ^{125}I. This iodinated thrombin maintained full factor VIII activating activity. A time dependent binding of factor VIII and thrombin was observed following chromatography on Bio-Gel A 1.5M. This observation, plus the absence of activation of factor VIII by solid phase thrombin, suggests that a factor VIII-thrombin complex rather than limited proteolysis is the mechanism of factor VIII activity enhancement by thrombin.

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