

DEVELOPMENT OF MONOSPECIFIC ANTIBODIES TO HUMAN FACTOR IX. Cheryl Y. Tiarks, Chin-Hai Chang and Liberto Pechet. University of Massachusetts Medical Center, Worcester, Massachusetts, U.S.A.

The purpose of this research was to develop neutralizing and precipitating antibodies to factor IX. Human factor IX, purified by the method of Rosenberg et.al. (J.Biol.Chem. 250:8883, 1975), was electrophoresed on acrylamide gel. Two major bands migrating adjacently were eluted. They contained factor IX activity only. The eluates and their homogenized gel segments 7 and 8 were injected separately into two rabbits, R1 and R2, respectively. On immunodiffusion the antiserum R1 showed one precipitating line with normal plasma. It neutralized human factor IX (20 Bethesda units) and also slightly neutralized factor X. It had no effect on factors II and VII. Following absorption of this antiserum with purified factor X it neutralized factor IX only. With continuous immunization, however, this antiserum revealed two new precipitating contaminants. The antiserum R2 neutralized only factor IX; it reached 220 Bethesda inhibitory units. On immunodiffusion it showed two precipitating lines, one of which disappeared after absorption with human albumin. On immunodiffusion and Laurell immunoelectrophoresis, the albumin-absorbed R2 antiserum showed one precipitin line of identity, or one rocket, with normal plasma, a Red Cross factor IX preparation (rich in factors IX, II and X), the original eluates 7 and 8, and a Hemophilia-B antigen-positive plasma. No line or rocket developed with normal plasma absorbed with aluminum hydroxide or with antigen-negative Hemophilia-B plasma. We conclude that the antisera R1 and R2 contain factor IX neutralizing antibodies and that albumin-absorbed R2 has monospecific precipitating antibodies to human non-activated factor IX.

DETECTION, PURIFICATION AND CHARACTERIZATION OF FACTOR IX-LIKE MOLECULES. R.M. Bertina and J.J. Veltkamp. Hemostasis and Thrombosis Research Unit, Department of Medicine, University Hospital, Leiden, The Netherlands.

An antibody specifically precipitating human factor IX has been raised in rabbits using highly purified factor IX (batchwise DEAE-Sephadex adsorption and elution, chromatography on heparin-Sepharose, disc-polyacrylamid gel electrophoresis; MW(SDS), 67,000).

With an electro-immuno-assay for factor IX 6 hemophilia B⁺ families were detected (only one of which showed a prolonged prothrombin time with ox-brain thromboplastin). All factor IX like material demonstrated normal electrophoretic mobility (when mixed with normal plasma) under standard conditions.

From one patient (hemophilia B_M) the factor IX-like material has been purified at least 3000 times and compared with normal factor IX. The factor IX-like material from all B⁺ families has been compared with normal factor IX with respect to their behaviour in 2-dimensional electrophoresis at different time intervals after the initiation of contact-induced coagulation. The precipitation peak of normal factor IX gradually disappears after activation. Improvement of the precipitation conditions during electrophoresis by the addition of polyethylene glycol at least partially prevents this phenomenon.

Finally, it is concluded that the use of a precipitating antibody against factor IX is a valuable tool in the study of factor IX-like molecules.

ACTIVATION OF HUMAN FACTOR X. Carolyn L. Orthner, Sam Morris and David P. Kosow. American National Red Cross Research Laboratory, Bethesda, MD, U.S.A.

Factor X is the zymogen of the proteolytic coagulation enzyme Factor Xa. Since the activation of Factor X to Factor Xa may be a rate limiting step of the coagulation cascade we have begun investigations of the mechanism of this reaction. Human Factor X has been purified 6000-fold from human plasma and the final product is over 95% pure as judged by polyacrylamide gel electrophoresis. Human Factor X has a monomeric molecular weight of 75,000 and consists of two chains held together by a disulphide bridge. The molecular weight of the heavy chain is 56,000 and that of the light chain is 17,500. The venom coagulant protein of *V. russelli* (RVV-X) activates human Factor X by cleaving the heavy chain. When fully activated, human Factor Xa shows two bands on polyacrylamide gel electrophoresis indicating that human Factor Xa like the bovine enzyme has two molecular forms.

The kinetic mechanism of the activation reaction has been investigated utilizing the chromogenic Factor Xa substrate Bz-Ile-Glu-Gly-Arg-p-Nitroanilide (S-2222). The reaction has an absolute requirement for Ca; Mg cannot substitute for Ca, however Mg can increase the Vmax of Xa formation in the presence of suboptimal concentrations of Ca. Both Ca and Mg effects exhibit positive cooperativity. Our data indicate that human Factor X has at least three cooperative metal binding sites some of which are specific for Ca.