

THE EFFECT OF METAL IONS ON HUMAN FACTOR IX ANTIGENICITY. R.M. Lewis, H.M. Reisner, B.C. Abels, Jr. and H.R. Roberts, Depts. of Pathology and Chemistry, University of North Carolina, Chapel Hill, NC, USA.

Affinity chromatography of an inhibitor to human factor IX (F.IX) separated the antibody into two populations. The ion dependent population of antibodies had an absolute divalent cation (Me^{++}) binding requirement. The non-ion dependent population bound F.IX equally in the presence or absence of Me^{++} . The concentration of Me^{++} required for $\frac{1}{2}$ the maximum ion dependent antibody binding ($\frac{1}{2}$ max) was (in mM) Ca^{++} 0.40, Mn^{++} 0.05, Sr^{++} 0.70 and Mg^{++} 0.65.

Ca^{++} potentiated the binding of antibody in the presence of excess Mg^{++} . In addition, the $\frac{1}{2}$ max for Ca^{++} was reduced about four fold. These observations are consistent with separate binding sites on the F.IX molecule for Ca^{++} and Mg^{++} and potentiation of Ca^{++} binding by Mg^{++} . Scatchard analysis of ion dependent antibody binding indicates about a 10 fold greater affinity of antibody in the presence of Ca^{++} than Mg^{++} . In the presence of both cations, affinity was at least as high as in the presence of Ca^{++} alone supporting the presence of separate ion binding sites on the F.IX molecule.

0420

USE OF POLY(L-LYSINE)-SEPHAROSE 4B FOR ISOLATION OF VITAMIN K DEPENDENT FACTORS FROM HUMAN PLASMA.

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Poly(L-lysine) (PLL; $M_r=15,000-40,000$) or human albumin was conjugated with Sepharose 4B-CNBr to obtain PLL-Sepharose 4B or albumin Sepharose 4B. When human citrated plasma was passed through a column (3x1 cm) of PLL-Sepharose 4B, it was observed that eluted plasma had a prolonged PTT time and further analysis showed that it was completely deficient in factors II, VII, IX, and X. Some loss of factors V, VIII, XI and XII was also noticed. Plasma passed through a column of albumin under identical conditions suffered no detectable loss of factors II, VII, IX and X. Loss of factors V, VIII, XI and XII was also reduced. Factors II, VII, IX and X that adsorbed to PLL-Sepharose 4B column did not elute from the column even after extensive washing (100 ml of 0.15 M NaCl containing 0.018 M sodium citrate in 5 ml aliquots). Immunoelectrophoretic examination of the eluate showed that it was essentially free of albumin indicating that the washing of the gel was satisfactory. Also, no other protein was detectable in the wash. Addition of 0.75 M NaCl containing 0.018 M sodium citrate to the washed gel resulted in the elution of factors II, VII, IX and X. Addition of the high salt eluate (after adjustment of ionic strength to 0.15 M NaCl with distilled water) to the plasma previously made deficient by passage through the PLL-Sepharose 4B column resulted in normalization of the PTT time. Subsequent analysis of the high salt eluate revealed that the recovery of various factors was: II=88%, VII=81%, IX=79% and X=92%. Preliminary studies indicated also that the eluate from PLL-Sepharose 4B was apparently devoid of activated factor X. The possibility of other activated blood clotting factors being present in the PLL-Sepharose 4B eluate is currently under investigation.

0419

ATYPICAL RESPONSE TO INFUSION PRIOR TO THE IN VITRO APPEARANCE OF INHIBITOR IN A HEMOPHILIA B PATIENT. C.H. Miller and M.W. Hilgartner. Department of Pediatrics, New York Hospital-Cornell University Medical Center and Division of Rheumatology, Hospital for Special Surgery, New York, N.Y., U.S.A.

Infusion studies were performed on six patients with severe hemophilia B, in whom factor IX clotting activity (IX:C) was less than 1%. All had no detectable factor IX antigen (IX:Ag) on testing with heterologous anti-IX prior to the study (and no measurable F. IX inhibitor). Each patient received the same factor IX concentrate in a 50 u/kg dose. Samples were drawn at 0, 15 minutes, 1 hour, 4 hours, and 24 hours post-infusion and were tested for IX: C and IX:Ag. Five of the patients showed similar response to transfusion with average half-life of IX:C of 12 hours and of IX:Ag 10 hours. One patient showed quite different results. He had a half-life of IX:C of 31 hours and of IX:Ag of 24 hours. Although routine tests for a F. IX inhibitor were negative both before and after the study, he developed a rapidly rising inhibitor 15 months later. In retrospect it can be seen that this patient resembled the patient with a low titer inhibitor and prolonged IX:Ag survival described by Goodnight et al. However, our patient showed the atypical infusion response pattern before his inhibitor was detectable in vitro. Thus the finding of a prolonged survival of IX:Ag can be taken as evidence of inhibitor formation. Inhibitor presence can be confirmed by detection of circulating immune complexes containing IX:Ag prior to the appearance of a measurable inhibitor to clotting.

0421

A SIMPLE METHOD FOR PREPARATION OF NONTHROMBOGENIC PROTHROMBIN COMPLEX. M. Wickerhauser. American Red Cross Blood Services Laboratories, Bethesda, Md., USA

Prothrombin complex concentrate (PCC) when infused into patients may produce thrombotic episodes, presumably due to the presence of some thrombogenic material. In order to prepare a safer PCC we have investigated whether the addition of purified antithrombin III (AT III) during an early stage of the isolation process would be effective in reducing thrombogenicity as assessed by the Wessler stasis model. By our method the cryosupernatant of plasma is adsorbed with DEAE-Sephadex (1.5 g/L), the unadsorbed fraction removed, the DEAE-cake washed with 0.7-1.0 plasma volume of citrate saline, followed by elution with 0.1 plasma volume of 0.03 M citrate containing 2 M NaCl and 0.25 AT III unit/ml, at pH 6.8. The eluted PCC is then concentrated and desalted by ultrafiltration, filtered, dispensed and lyophilized.

In a bench-scale experiment starting from 6 L cryosupernatant, a PCC was obtained at 43% recovery and 50-fold purification over plasma based on Factor IX activity. The recoveries of Factors II, VII and X were 44, 23 and 64% respectively. The concentrate was free of detectable thrombin and plasmin and contained about 1 unit of AT III (determined immunologically) per 25 units of Factor IX. In the Wessler stasis model this PCC was nonthrombogenic at 100 Factor IX units per kilo body weight of the rabbit. Higher doses were not tried. A control PCC prepared without the addition of AT III to the DEAE-eluting buffer was nonthrombogenic at 50, but thrombogenic at 75 Factor IX units per kilo body weight of the rabbit. Subsequent incubation with AT III did not eliminate this thrombogenicity.

These results suggest that AT III can be effective in preventing thrombogenicity in PCC but not in abolishing it if already developed.