HETEROGENEITY OF FACTOR IX IN THERAPEUTIC CONCENTRATES. D. Ménaché and D. Aronson. Hôpital Beaujon, Clichy, France ; Bureau of Biologics, Bethesda, Maryland, U.S.A.

In vivo and in vitro experience shows that factor IX concentrates are partially activated. A rabbit antibody to human factor IX was used to investigate the factor IX antigenic content and electrophoretic mobility of commercial products as well as experimental "activated products".

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Rocket immunoelectrophoresis of all concentrates showed a 1.5-3 fold increased antigenic content/unit factor IX clotting activity when compared to plasma. Two dimensional crossed immunoelectrophoresis of standard factor IX preparation produced a single sharp peak whether electrophoresed in Ca or EDTA containing buffer. "Activated" preparations produced a dome shaped precipitin arc. The addition of plasma to factor IX concentrates yielded a marked shoulder only when the electrophoresis was done in EDTA. This effect could not be reproduced by the addition of antithrombin III (AT-III). The addition of plasma to the activated IX (IXa) revealed an even more pronounced heterogeneity whether in Ca or EDTA. The addition of AT-III produced a second precipitin peak when activated IX was electrophoresed in the presence of Ca^{++} .

These results indicate that at least three forms of factor IX exist in factor IX preparations. The absence of detectable AT-III reacting material in the regular IX preparations is a priori evidence of the absence of major amounts of IXa, whereas the presence of AT-III reacting material in the "activated" complex is evidence of biologically active material.

CIRCULATING FACTOR IX ANTIGEN-INHIBITOR COMPLEXES IN HEMOPHILIA B FOLLOWING INFUSION OF A FACTOR IX CONCENTRATE. S.H. Goodnight, C.W. Britell, K.D. Wuepper and B. Østerud. University of Oregon Health Sciences Center, Portland, Oregon and University of California at San Diego, San Diego, California, U.S.A.

A persistent low titer IgG factor IX inhibitor (0.8-2.3u/ml) has been previously described in an 11 year old boy with severe hemophilia B-. Since the disappearance of factor IX antigen (IXAGN) following Konyne(R) infusion was markedly delayed ($T_{1/2}$ 60 hr) compared to factor IX clotting activity ($T_{1/2}$ 8 hr) (Blood 48:977, 1976), a search for circulating factor IX antigen-inhibitor complexes was undertaken. The inhibitor binds firmly to staphylococcal protein A - Sepharose 4B (SPA-Sepharose) but may be eluted in the IgG 1, 2, and 4 fraction with 3M NaSCN. SPA-Sepharose chromatography of post-infusion plasma (IXAGN 2.7u) from the patient with the inhibitor showed IXAGN (0.5u) in the IgG 1, 2, and 4 fraction, whereas IXAGN was not found in that fraction of chromatographed samples of post infusion plasma from non-inhibitor patients with hemophilia B- or B+. Using a highly specific antibody to factor IX, two dimensional immunoelectrophoresis of purified factor IX or normal plasma showed a single symmetrical fast moving peak. When an equal volume of inhibitor plasma was added to either factor IX or normal plasma a second, slower moving component was also seen. Two dimensional immunoelectrophoresis of plasma obtained from the patient 45 minutes after infusion showed both the slow and fast components. The presence of IXAGN in the IgG 1, 2, and 4 peak after SPA-Sepharose chromatography and the demonstration of 2 components on two dimensional immunoelectrophoresis indicates the presence of circulating factor IX antigen-inhibitor complexes in the plasma of this patient following the infusion of a factor IX concentrate.

FACTOR Xa LIKE ENZYME IN SNAKE "HABU" VENOM. I. Maruyama, T. Kinoshita*, M. Kazama*, A. Igata and T. Abe*. Kagoshima University School of Medicine, Kagoshima and Teikyo University School of Medicine*, Itabashi, Tokyo, Japan.

The snake "HABU" venom was divided into subfractions and their influence on both platelet and coagulation were studied. Four fractions were obtained by gel exclusion chromatography on Sephadex G75 of which molecular weight ranged from 100,000 to 5,000. The fraction of the largest molecular weight was further divided into four subfractions; I-1, I-2, I-3 and I-4 by DEAE cellulose chromatography. The subfraction I-1 showed no activity to induce platelet aggregation but suppressed ADP, collagen and ristocetin-induced aggregation. The subfraction I-2 and I-3 equally induced the aggregation of plasma platelet and gel filtrated platelet suspension. The subfraction I-4 had the same effect on the platelet aggregation except for the specific suppressive effect on the ristocetin aggregation. The subfraction I-3 was found to have Xa like activity which was revealed using chromogenic substance (S-2222). This activity was not inhibited by purified human antithrombin III, α_2 -macroglobulin or α_1 -antitrypsin. The relationship between activities of these fractions on platelet and coagulation suggested that they are different from any known coagulation factor.

HABU--- Trimeresurus flavoviridis Hallowell