ACTIVATION STUDIES ON A HUMAN PROTHROMBIN VARIANT : PROTHROMBIN BARCELONA. R. Benarous°, M.J. Rabiet+, D. Labie° and F. Josso+.° Institut de Pathologie Moléculaire, CHU Cochin, 75014 Paris, + Département d'Hématologie, CHU Necker Enfants-Malades, 75015 Paris (France).

Prothrombin Barcelona tested in the plasma from homozygous patients is characterized by a functional defect (5 % activity by a one-stage assay) contrasting with a normal prothrombin concentration found by immunochemical assay.

This low biological activity is the expression of a very slow rate of conversion of prothrombin into thrombin. It may be postulated that the abnormal kinetics of conversion of this variant might involve an inhibition of one of the proteolytic cleavages responsible for the activation process. To test this hypothesis, purified normal and abnormal prothrombins were submitted to proteolysis with factor Xa and thrombin, either with or without protease inhibitors. Prothrombin cleavage was checked at various times by SDS gel electrophoresis.

In the presence of factor Xa, conversion of normal prothrombin into intermediate II started within 30 min and was almost complete within two hours. In the same conditions, conversion into intermediate II of prothrombin Barcelona was only initiated after two hours. Conversely upon treatment of both prothrombins with thrombin, a slight delay in the formation of intermediate I was observed with prothrombin Barcelona, this formation being nevertheless complete after one hr.

In conclusion, the abnormal activation of prothrombin Barcelona seems mainly due to a decreased and delayed formation of intermediate II.

CHARACTERIZATION OF FACTOR VIII-INHIBITOR BY-PASSING ACTIVITY (FEIBA). K. Hess, N. Shih, and G. Tishkoff. American Red Cross, Lansing, Michigan, U.S.A.

In an attempt to identify the thrombogenic factor in human factor IX concentrates, we have studied the role of trace quantities of activated clotting factors employing an assay that compares the Factor VIII-like activity of IX concentrates with the ability of these products to restore to normal the abnormal activated partial thromboplastin time (APTT) of Factor VIII inhibitor plasma after 1 minute and 40 minute incubation. A coagulant activity (FEIBA) was evident when partially purified Factors X and II were combined in vitro. Factor Xa (4×10^{-4}u) plus Factor II gave negative results. Factor IIa (5.5×10^{-2}u), when combined with Factor X, generated FEIBA. Activated clotting factors (Xa, IIa) when tested alone, at comparable levels, were devoid of FEIBA. Our results suggest a mechanism, distinct from activated clotting factors, that can effectively by-pass the Factor VIII defect in the coagulation cascade. The proposed mechanism appears to also by-pass the normal inhibitory properties (i.e., antithrombin III) of human blood.

DETECTION OF ACQUIRED INHIBITORS OF FACTOR IX WITH PRECIPITATING RABBIT ANTISERA AGAINST FACTOR IX. K.H.Ørstavik, Institute of Medical Genetics, University of Oslo, Norway and I.M.Nilsson, Allmänna Sjukhuset, Malmø, Sweden.

The effect of acquired inhibitors of factor IX on factor IX antigen was tested in a modified electroimmunoassay with a rabbit antiserum against factor IX. Plasma from three patients with hemophilia B and inhibitor against factor IX was mixed with agarose and casted in a one cm high intermediate gel between the sample wells and the agarose gel containing the rabbit antiserum. The precipitation of factor IX antigen by the rabbit antiserum was prevented when factor IX antigen migrated through the intermediate gel containing plasma from two of the patients with inhibitor titre 7.9 and 0.8 U/ml. The concentration of inhibitor plasma necessary to reduce by 50% the amount of precipitated factor IX antigen from 10 µl normal plasma could be determined for the two patients and was approximately 2% for the patient with titre 7.9 and 15% for the patient with titre 0.8. The third patient had a titre of 0.13 U/ml and only a slight reduction in precipitated antigen was obtained with a high concentration of plasma in the intermediate gel (30%). The precipitation of factor IX antigen from 7 patients with hemophilia B+ (factor IX antigen 17-109%) and one patient with hemophilia B_M (factor IX antigen 98%) was also inhibited by plasma from the two patients with inhibitor titre 7.9 and 0.8. This study suggests that absorption in intermediate gel may be a simple technique for the detection of inhibitors with titre 0.8 and higher and also for the study of the specificity of inhibitors.