PRIMARY STRUCTURE OF BOVINE FACTOR IX. K. Titani, D. L. Enfield, K. Katayama, L. H. Ericsson, K. Fujikawa, K. A. Walsh and H. Neurath. Department of Biochemistry, University of Washington, Seattle, Washington, U. S. A.

Factor IX is the zymogen of a serine protease which participates in the middle phase of intrinsic blood coagulation. Bovine factor IX is a single chain glycoprotein with a molecular weight of 55,000. Upon activation by factor XI_a(activated factor XI), cleavage of two specific peptide bonds (Arg-Ala and Arg-Val) gives rise to factor IX_a(activated factor IX) having a two chain structure(mol wt 46,000) and an activation peptide(mol wt 9,000)(K. Fujikawa, M.E. Legaz, H. Kato and E. W. Davie, Biochemistry, 13, 4508 (1974)). A protease from Russell's viper venom (RVV) also activates factor IX by cleaving only one specific peptide bond (Arg-Val)(P.A. Lindquist, K. Fujikawa and E. W. Davie, Fed. Proc., 35, 1353 (1976)). Bovine factor IX_a(RVV) thus activated has a molecular weight of 55,000 identical to that of factor IX but is composed of two peptide chains held together by one or more disulfide bonds. After reduction and alkylation of disulfide bonds, two peptide chains were separated on a column of SP-Sephadex C-25 by a salt and pH gradient in the presence of 7 M urea. The sequence analysis of factor IX is in progress on these two peptide chains using their subpeptides produced by cleavage at methionine, lysine, arginine and tryptophan. Preliminary results indicate that the sequence of factor IX is homologous to that of factor X and related also to prothrombin and other vitamin K-dependent blood coagulation factors. A high degree of sequence homology is also observed between the heavy chain (the carboxyl-terminal half of the molecule) of factor IX_a and pancreatic serine protease such as trypsin, chymotrypsin and elastase.

GENETIC VARIANTS OF HEMOPHILIA B. H.R. Roberts, K.S. Chung and J.C. Goldsmith. University of North Carolina School of Medicine, Chapel Hill, North Carolina, U.S.A.

The purpose of this report is to describe genetic variants of hemophilia B. Most variants have been distinguished on the basis of clinical severity of their disease, as well as immunological, functional, and biochemical characterization of the Factor IX molecule. They have been classified according to the degree of cross-reactivity of the Factor IX molecule with specific homologous and heterologous anti-Factor IX antibodies using both inhibitor neutralization and radioimmunoassay techniques. Some hemophilia B variants have a Factor IX molecule that cross-reacts completely with anti-Factor IX antibodies. Other hemophilia B patients have a Factor IX molecule that has no detectable cross-reaction with anti-Factor IX antibodies. Still other hemophilia B patients show reduced levels of cross-reacting material. We have extensively studied one hemophilia B variant with a Factor IX molecule that shows complete cross-reaction with anti-Factor IX antibody. The Factor IX from this patient has been isolated and has been shown to have 5% clotting activity and 100% antigenic activity. This variant Factor IX molecule shows delayed activation in the presence of partially purified Factor XI_a and Ca^{2+} although it is otherwise similar to normal human Factor IX in terms of molecular weight and the number of γ-carboxyglutamic acid residues per molecule. Other Factor IX variants have also been characterized and will be discussed. For example, hemophilia B_M variants, unlike the usual type of hemophilia B, show prolonged ox-brain prothrombin times. These patients have an abnormal Factor IX molecule that is recognized antigenically, but not functionally.

HEMOPHILIA B: GENETIC VARIANTS AND CARRIER DETECTION. C.K.Kasper,B.Østerud,S.I.Rapaport.School of Medicine,Univ. of Southern California;Orthopaedic Hospital,Los Angeles; School of Medicine, Univ. of California at San Diego; V.A.Hospital,San Diego; U.S.A.

In 92 males with hemophilia B from 71 kindreds,we measured factor IX activity,prothrombin time using bovine thromboplastin (bovine tpln time),and factor IX antigen both by inhibitor neutralization using a human factor IX inhibitor and by electroimmunoassay using a precipitating rabbit anti-human-factor IX antiserum. Eighty patients with 3% or less factor IX activity could be divided into 4 groups:(1) 7 patients with greatly prolonged bovine tpln times and normal levels of factor IX antigen;(2) 17 patients with mildly prolonged bovine tpln times and factor IX antigen levels between about 25% and normal;(3) 8 patients with normal bovine tpln times and antigen levels between about 25% and normal; (4) 48 patients with normal bovine tpln times and no measureable antigen excess. Some of the latter group were also tested with a chicken anti-human-factor IX antiserum and no antigen was found. None of 12 patients with mild hemophilia B (factor IX activity of 4 to 22%) had a prolonged bovine tpln time although 4 patients had excess factor IX antigen over activity. Thus,about 1/3 of these 92 hemophilia B patients had evidence of an abnormal factor IX molecule. Factor IX activity was also measured in 48 normal women and in 51 definite carriers of severe hemophilia B. Probability curves were derived to estimate the chance of a woman being a carrier based upon her factor IX level and her degree of kinship to a definite carrier. The relation between factor IX activity and antigen was also delineated for normal women and for carriers. In kindreds in which affected males had excess antigen, some carriers could be distinguished from normal women on the basis of excess antigen over activity.In appropriate kindreds, prolonged bovine tpln times helped distinguish some carriers.