ISOLATION AND PROPERTIES OF THE ABNORMAL FACTOR IX MOLECULE OF HEMOPHILIA B_M. B. Østerud, K. Lavine, C.K. Kasper, and S.I. Rapaport. University of California and V.A. Hospital, San Diego, California, and Orthopaedic Hospital, Los Angeles, California, U.S.A.

Abnormal factor IX from a hemophilia B_m patient (F.IX-B_m) has been isolated to homogeneity on SDS polyacrylamide gel electrophoresis by the same technique utilized for purifying

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normal F.IX. F.IX-B_m generated no measurable procoagulant activity when incubated with F.XI_a in a two-stage F.IX_a assay (normal F.IX, 55 sec; F.IX-B_m, >30 min). F.IX-B_m inhibited the activation of F.X by F.VII and ox brain thromboplastin as measured in an amidolytic assay for factor X_a. Normal F.IX also inhibited this reaction but to a five times lesser degree. F.IX-B_m has the same molecular weight on SDS gel electrophoresis as normal F.IX (55,000) and does not differ from normal F.IX in its amino acid composition. F.XI_a cleaves F.IX-B_m in the presence of Ca ions at the same rate as it cleaves normal F.IX, yielding a heavy chain of 27,000 molecular weight and a light chain of 16,000 molecular weight. However, the cleavage does not give rise to procoagulant activity. Like normal F.IX_a, the cleaved forms of F.IX-B_m appear to bind phospholipid since F.IX-B_m protein was precipitated with phospholipid in the presence of Ca ions. These data support an hypothesis that the abnormality in the F.IX-B_m molecule stems from a defect at the active site.

PROTHROMBIN BIOSYNTHESIS: THE VITAMIN K-DEPENDENT CARBOXYLASE. J. W. Suttie, S. R. Lehrman, D. H. Rich, and D. S. Whitlon. University of Wisconsin-Madison, Madison, Wisconsin, U.S.A.

We have shown that the pentapeptide Phe·Leu·Glu·Glu·Val serves as a substrate for a vitamin K-dependent liver microsomal carboxylase that converts peptide-bound glutamyl residues to γ-carboxyglutamyl residues. Requirements of the solubilized microsomal system for peptide carboxylation have been determined and are similar to requirements for prothrombin precursor carboxylation. Additional peptides have now been synthesized, and the available data suggest that specificity of the vitamin K-dependent carboxylase involves both macromolecular and active-site recognition sequences. Inhibition of the *in vitro* carboxylation system by the anticoagulant Warfarin has now been studied in detail. Dithiothreitol stimulates carboxylase activity and replaces the requirement for NADH as a reductant needed to form the hydroquinone. Warfarin is a potent inhibitor of the DTT driven carboxylase reaction in intact microsomes, but a much poorer inhibitor when NADH is used as a reductant. In a microsomal system with DTT (but not NADH) as a reductant, vitamin K epoxide is an active form of the vitamin. In a Triton X-100 solubilized system; NADH is required, Warfarin is ineffective as an inhibitor, the epoxide is not an active form of the vitamin, and the conversion of vitamin K epoxide to the vitamin cannot be demonstrated. These data support the hypothesis that Warfarin inhibits vitamin K activity through an effect on epoxide reduction and suggests that this reaction is driven by a nonpyridine nucleotide pathway which, *in vitro*, can use DTT instead of an unidentified physiologically active reducing agent.

SURVIVAL OF RADIOLABELLED HUMAN FACTOR VIII IN VIVO. J.Over, J.J.Sixma, A.M.C.Trieschnigg, H.A.A.Vlooswijk, M.H.M.Doucet-de Bruïne, N.Beeser-Visser and B.N.Bouma. Dept.of Haematology, University Hospital Utrecht, The Netherlands.

Bennett and Ratnoff(1972) reported that in haemophiliacs the coagulant activity of factor VIII(F VIII:C) disappeared faster than the factor VIII-related antigen (F VIIIR:AG).We decided to investigate this phenomenon with radiolabelled factor VIII.This also allowed us to study the behaviour of various factor VIII fractions after infusion.Human factor VIII was purified from cryoprecipitate,labelled with 125 I by the lactoperoxydase-glucose oxydase method,mixed with a human albumin solution and sterilized by filtration through a Millipore filter. F VIII:C,F VIIIR:AG,F VIIIR:WF assays,gelchromatography,crossed immunoelectrophoresis and PAGE studies were carried out.No significant changes due to the procedure were observed.The radiolabel in the plasma after in vivo administration was for more than 95% bound to F VIIIR:AG as demonstrated with immunoadsorption with an antiserum prepared against a subunit of factor VIII purified by PAGE.About 1.5-6% of free 125 I was also present.Yield and survival were studied in 6 normal volunteers.The yield was somewhat lower (79%) than expected after transfusion.The survival curve was biphasic showing half lives of 4½ hours and 20 hours.In 4 haemophiliacs the yield and disappearance were essentially similar.The F VIII:C in these patients who also received cryoprecipitate of 20 donors disappeared with the same kinetics as the radiolabel.Evidence was obtained indicating that the highest molecular weight forms of factor VIII disappeared more rapidly,while losing the F VIIIR:WF,than the lower molecular weight forms.Radiolabel in cryoprecipitate showed a biphasic,more rapid disappearance;label in cryosupernatant plasma disappeared slower.