SIGNIFICANT ROLE OF FRAGMENT 1•2 PLUS LIGHT CHAIN OF BOVINE HIGH MOLECULAR WEIGHT KININOGEN IN CONTACT MEDIATED COAGULATION. R. Waldmann, A.G. Scicli, G.M. Scicli, J.A. Guimaraes, O.A. Carretero, H. Kato, Y.N. Han and S. Iwanaga, Henry Ford Hospital, Detroit, Michigan, USA, and Institute for Protein Research, Osaka University, Osaka, Japan.

We have previously reported that high molecular weight kininogen (HMWK) partially corrects the partial thromboplastin time (aPTT) of Fitzgerald Trait plasma (HMWK deficiency), and in addition, we have also reported that kinin free HMWK which is also Fragment 1•2 free has decreased correcting capability. Presently, we have studied the effect of lysyl-bradykinin-free HMWK with Fragment 1•2 intact, obtained by hydrolysis of HMWK with urinary kallikrein. In addition, the effect of light chain with and without attached Fragment 1•2 were studied. When the correcting capacity of HMWK was compared, on a molar basis with the above-mentioned fragments, the following results were obtained. Kinin and Fragment 1•2 free HMWK has a significantly decreased correcting capacity on the aPTT of Fitzgerald trait plasma while lysyl-bradykinin free HMWK shows no decrease in correcting capability. Light chain with Fragment 1•2 attached also has intact correcting capability while light chain alone has a significantly decreased correcting activity. The above data suggest that the active site of HMWK resides in the light chain with Fragment 1•2 attached. Since Fragment 1•2 alone has inhibitory capacity, it may serve as a binding site and the light chain as the active site.

ACTIVATION OF HAGEMAN FACTOR BY FACTOR XI_A-HMW KININOGEN. Henry L. Meier, Russell E. Thompson, and Allen P. Kaplan. National Institutes of Health, Bethesda, Md.

Plasma deficient in prekallikrein possesses an abnormality in the kinetics of surface dependent initiation of coagulation and fibrinolysis. This defect appears to be secondary to a diminished rate of Hageman factor activation, however the abnormality is progressively diminished as the time of incubation of the plasma with appropriate surfaces is increased. Since factor XI and prekallikrein circulate bound to HMW kininogen and HMW kininogen is known to augment the activation of factor XI and prekallikrein by activated Hageman factor, the ability of factor XI_A to activate Hageman factor was examined. One ug Hageman factor was bound to supercel alone, or in the presence of 1.5 ug HMW kininogen and 0.1 to 1.0 ug factor XI_A for varying time periods (0-60 min). The pellet was washed X 3 and bound activated Hageman factor was assayed by its ability to convert prekallikrein to kallikrein. The kallikrein generated was quantified by release of p-nitroaniline from α-benzoyl phe-val-arg-p-nitroanilide. Factor XI_A alone was a weak activator of Hageman factor and the quantity of kallikrein generated was augmented when HMW kininogen was included in the incubation mixture. With limited HMW kininogen the Hageman factor activity appeared proportional to the factor XI_A added. The same result was obtained with factor XI_A isolated from prekallikrein deficient plasma. The data suggest that factor XI_A plus HMW kininogen may represent one of the additional feedback mechanisms by which Hageman factor may be activated and thereby contribute to the gradual activation observed in prekallikrein deficient plasma.

MECHANISM OF ACTIVATION OF HUMAN FACTOR IX BY ACTIVATED HUMAN FACTOR XI. Bjarne Østerud, Bonno N. Bouma and John H. Griffin. Department of Medicine, University of California and Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California, U.S.A.

Human Factor IX (F.IX) has been highly purified using: $BaSO_4$ adsorption and elution, DEAE-cellulose chromatography, preparative polyacrylamide gel electrophoresis, and chromatography on heparin-agarose. The purified F.IX appeared homogeneous on SDS-gels and revealed a single polypeptide chain of 55,000 MW in the Weber-Osborn system. Human Factor XI was purified by ion exchange chromatography to greater than 95% homogeneity as judged on polyacrylamide gels. Following activation of Factor XI by purified human Factor XII_a, the Factor XI_a was a potent activator of F.IX in a Ca^{++}-dependent reaction. As judged on SDS gels, activated F.IX at 43,000 MW contains two disulfide-linked polypeptide chains of 27,000 and 16,000 MW. These MW values for the zymogen and activated form of F.IX are identical to those reported for bovine F.IX. And the amino acid compositions of human F.IX and of the isolated heavy and light chains of $F.IX_a$ are quite similar to those reported for bovine F.IX and $F.IX_a$. Based on these observations, it is suggested that activated human F.IX contains its active site in a C-terminal 27,000 MW polypeptide which is linked by disulfide bonds to a 16,000 MW N-terminal polypeptide containing gamma carboxyglutamic acid.