RAPID LOSS OF FACTOR XII- AND XI-ACTIVITY IN ELLAGIC ACID ACTIVATED NORMAL PLASMA: ROLE OF PLASMA INHIBITORS AND IMPLICATIONS FOR AUTOMATED APTT-RECORDING. J.H. Joist, J.F. Cowan and M. Khan. Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Rapid prolongation of the activated partial thromboplastin time (aPTT) of normal plasma upon incubation with ellagic acid containing aPTT reagents was observed. The aPTT prolongation was not due to time dependent changes in pH in the incubation mixtures or loss of activity of the labile coagulation factors VIII and V, but occurred as a result of rapid progressive inactivation of ellagic acid activated factors XII and XI. Prolongation of the aPTT and loss of contact factor activities was not observed in plasma incubated with particulate activator reagents. This finding seemed to indicate that adsorption of factors XII and XI to larger particles during the activation process may protect these factors from inactivation by naturally occurring plasma inhibitors. Evidence is presented which supports previous observations that C1-inhibitor, $\alpha_1$-antitrypsin and antithrombin III (in the presence of heparin contribute to factor XIIa- and XIa-inactivation in ellagic acid activated plasma and that plasma albumin may compete with factor XII for ellagic acid binding. The findings indicate that ellagic acid containing aPTT reagents have unfavorable properties which seriously limit their usefulness in the clinical laboratory, particularly in respect to recording of the aPTT with certain fully automated clot timers.

THROMBIN GENERATION THROUGH THE SHORT-CIRCUIT XII-XI-VII. Altman, R. and Colodner, C. U.A.M.I. Rivadavia-Peralta Ramos, Departamento de Investigación, Buenos Aires, Argentina.

Altman and Hemker reported the activation of factor VII by factors XII and XI and it seems to us important to demonstrate the possibility of thrombin formation through this short-circuit between the intrinsic and extrinsic clotting system. Kaolin particles coated with human fibrinogen clumps by effect of thrombin. With concentrations of 0.1 U/ml or more, clumping was clearly visible. Hirudin prevent the clumping of the kaolin coated particles. When kaolin was incubated with citrated plasma, we supposed that fibrinogen is adsorbed on kaolin surface. Then, various deficient plasmas were tested. In plasmas deficient of factor XII, XI or II, none clumping were obtained. In factor VII deficient plasma, little degree of effect was observed. The addition of contact product (CP) to Hageman plasma returned its clumping capacity. This effect is not due to CP itself. $Ca^{++}$ seems to be necessary for this action because EDTA 3mEq, inhibit the clumping capacity of normal plasma. We concluded that factors XII, XI, VII, II and $Ca^{++}$ are absolutely necessary for the thrombin generation in these experiments, whereas factors IX, VIII, X and V are not.

INTERACTION OF FACTOR XI AND KALLIKREIN WITH HIGH MOLECULAR WEIGHT KININOGEN. Russell E. Thompson, Robert J. Mandle Jr. and Allen P. Kaplan. National Institutes of Health, Bethesda, Md.

The molecular weight (mol. wt.) of factor XI in normal plasma fractionated on Sephadex G-200 was over 400,000, while the mol. wt. of factor XI in HMW kininogen deficient plasma was 175,000. When HMW kininogen deficient plasma was made 70 µg/ml in HMW kininogen and fractionated on Sephadex G-200, factor XI was again found at mol. wt.~ 400,000. Prekallikrein was found complexed to HMW kininogen (Mandle and Kaplan PNAS 73: 4179, 1976) however, no complex containing both prekallikrein and factor XI was identified and neither Hageman factor nor plasminogen were complexed to HMW kininogen. Gel filtration of normal plasma had a major peak of HMW kininogen at mol. wt. of 200,000 while isolated HMW kininogen had a mol. wt. of 130,000 on reduced SDS gels (SDS-PAGE). Alkaline disc gel electrophoresis of HMW kininogen revealed two bands of equal intensity and each possessed the ability to correct the coagulation defect of HMW kininogen deficient plasma. Digestion of HMW kininogen with kallikrein generated bradykinin and a peptide of mol. wt. 13,000, while SDS-PAGE of the residual HMW kininogen revealed bands of mol. wt. 110,000 and 120,000. Upon reduction, bands at mol. wts. 77,000, 66,000, and 37,000 were obtained. Antibody specific for HMW kininogen reacted with either native or kallikrein-treated HMW kininogen but not reduced, kinin-free kininogen. Kinin-free kininogen, reduced kinin-free kininogen, and the major fragment eluted from alkaline disc gels after electrophoresis of reduced kinin-free kininogen all retained coagulant activity. Factor XI as well as prekallikrein therefore circulate complexed to HMW kininogen; these complexes may then interact with surface-bound Hageman factor. Kallikrein digests human HMW kininogen to yield bradykinin, a peptide, and disulfide-linked fragments which retain functional activity.