IDENTIFICATION OF A PROTHROMBIN DERIVATIVE IN HUMAN URINE. A. Bezeaud, D. Aronson, D. Ménaché and M.C. Guillin. Hôpital Beaujon, Clichy, France ; Bureau of Biologics, Bethesda, Maryland, U.S.A.

Urine samples of 13 normal subjects and 18 patients with constitutional coagulation defects were screened for the presence of prothrombin derivatives using specific rabbit antisera to human prothrombin, prothrombin Fragment 1 and Prethrombin 2. Patients comprised 15 severe and one moderate factor VIII deficiency, one factor VII deficiency and one mild factor XI deficiency. Immunological techniques included immunodiffusion, counter electrophoresis, immunoelectrophoresis, crossed immunoelectrophoresis and electroimmunoassay.

Antiprothrombin antiserum reveals in normal urine a prothrombin derivative which is in complete identity with Fragment 1, in partial identity with prothrombin and in non identity with Prethrombin 1. Other characteristics such as adsorption onto tricalcium phosphate and electrophoretic mobility are also those of Fragment 1. Similar results are obtained in the urine of patients with constitutional coagulation defects. Trace amounts of another prothrombin related antigen are also shown following concentration of normal urine.

These results indicate that prothrombin Fragment 1 is a constituent of human urine. Whether this constituent appears following glomerular filtration of a plasmatic Fragment 1 or following tubular degradation of prothrombin is not known.

A MOLECULAR MODEL FOR THE CALCIUM MEDIATED BINDING OF THE VITAMIN K DEPENDENT COAGULATION FACTORS TO PHOSPHOLIPID SURFACES. C.M. Jackson, F.A. Dombrose and C.W. Peng. Washington University School of Medicine, St. Louis, Missouri, USA.

Bovine Prothrombin Fragment 1 binding to phospholipid vesicles has been quantitatively characterized to provide a basis for understanding the calcium mediated binding of the vitamin K related proteins to phospholipids. Using equilibrium binding techniques, column chromatography, ultraviolet difference spectroscopy and analytical ultracentrifugation calcium binding to Fragment 1, calcium induced self association of Fragment 1 and calcium mediated binding of Fragment 1 to lipid vesicles has been characterized. Calcium induced self association of Fragment 1 explains the divergent results from various laboratories on calcium binding to this protein and is important in understanding binding of Fragment 1 to phospholipid. The number of Fragment 1 molecules binding to each phospholipid vesicle has been shown to be proportional to the number of negatively charged lipid molecules in the vesicle. At low calcium concentrations the affinity of Fragment 1 for lipid is dependent on calcium concentration; at higher concentrations binding becomes independent of calcium concentration. Binding is dependent on ionic strength implicating electrostatic contributions to the association. A model for calcium mediated binding of Fragment 1 to phospholipid which is consistent with calcium binding to the protein, calcium induced self association and calcium dependence of binding to lipid has been proposed.

ACTIVATION OF BOVINE BLOOD COAGULATION FACTOR V, A PREREQUISITE FOR IT TO BIND BOTH PROTHROMBIN AND FACTOR Xa. M.C. Guillin, A. Bezeaud, J.P. Freeman and C.M. Jackson. Hôpital Beaujon, Clichy, France ; Department of Biological Chemistry, Washington University, St-Louis, Mo, U.S.A.

It is known that prior to bind bovine prothrombin and to become fully functional, bovine Factor V must itself be "activated" by either thrombin or an enzyme isolable from Russell's viper venom. The purpose of this work was to determine if Factor V activation is also required in order for it to bind bovine Factor Xa.

This has been investigated by measuring the binding of both "native" (unactivated) Factor V and Factor V activated by the Russell's viper venom activating enzyme, to a column of agarose-bound Factor Xa. The experiments were also performed using diisopropylfluorophosphate (DFP) inhibited Factor Xa covalently bound to agarose. Both purified bovine Factor V (Va) and bovine plasma were used and gave the same results. In order to prevent initiation of clotting in bovine plasma, heparin was added to the plasma to promote inactivation of Factor Xa by antithrombin III.

The results indicate that Factor V activation is a prerequisite for it to bind Factor Xa ; Factor Va binds both Factor Xa and DFP inhibited Factor Xa, unmodified Factor V does not.

These experiments suggest that Factor V may not participate in prothrombin activation at all, until after some thrombin has been formed. If this is so, an alternate pathway by which the first thrombin is generated must be considered and may be proposed to be simply that involving Factor Xa, phospholipid and Ca^{2+} alone.