

STRUCTURAL CHANGES IN BOVINE FACTOR X ASSOCIATED WITH ACTIVATION. B. Furie and B.C. Furie, Tufts-NEMC and Tufts Univ. School of Medicine, Boston, MA, U.S.A.

Bovine Factor X is converted to  $X_A$  in the presence of Ca (II) by the coagulant protein of RVV. To monitor structural transitions in Factor X during conversion, the ultraviolet absorption, fluorescence emission, and circular dichroism spectra of  $X_A$  and Factor X were compared. The U.V. difference spectrum in the aromatic region comparing  $X_A$  and Factor X is characterized by differences due to tryptophan and tyrosine perturbations. The activation of Factor X yielded a time-dependent increase in this spectrum which was linear for about 60 min. and which paralleled the development of activated Factor X activity. The binding of Ca (II) to Factor X is associated with a red-shifted tryptophan difference spectrum; however, this perturbation makes only a small contribution to the total perturbation observed during Factor X activation. Solvent perturbation studies in 20% glycerol suggest that an average of 3.1 tryptophan residues and 9.0 tyrosine residues are exposed to solvent in Factor X; an additional 0.5 tryptophan residue and tyrosine residue become exposed to solvent during activation of Factor X. The activation of Factor X by the venom protein is associated with a small red shift in the intrinsic tryptophan fluorescence emission spectrum. Far- and near-U.V. circular dichroism spectroscopy detected no difference between Factor X and  $X_A$ . In summary, the activation of Factor X to  $X_A$  appears associated with exposure of tryptophan and tyrosine side chains previously buried within the protein and with minimal changes in the secondary structure. These results suggest that conversion of Factor X to activated Factor X involves functionally important, but structurally subtle, changes in the three-dimensional structure.

## INVITED SYMPOSIUM XII

### Comparative Hemostatic Mechanisms of Animals.

BLOOD COAGULATION IN THE HORSESHOE CRAB (*Limulus polyphemus*): A MODEL FOR MAMMALIAN COAGULATION AND HEMOSTASIS. Jack Levin, Johns Hopkins Univ. School of Medicine, Baltimore, Md., USA

The amoebocyte is the only type of circulating cell in the blood of *Limulus*. It is a nucleated cell, the cytoplasm of which is packed with granules. The coagulation system of *Limulus* is contained exclusively within the amoebocytes. Furthermore, factors necessary for blood coagulation are localized within their cytoplasmic granules. Cell-free plasma does not clot and is not required for coagulation. Aggregation and disruption of cells follow exposure of amoebocytes to foreign surfaces. Endotoxin produces similar changes and in addition, results in coagulation of the blood, following release from amoebocytes of the components of the coagulation mechanism. Coagulation, produced by endotoxin, is the result of activation by endotoxin of an enzyme (or series of enzymes) that in turn reacts with the clottable protein. Endotoxin does not react directly with the clottable protein. The rate of the reaction depends upon the concentration of endotoxin.

In both *Limulus* and mammals, disruption of the integrity of the circulatory system or exposure of blood to foreign surfaces results in aggregation of amoebocytes or platelets, with subsequent changes in shape, disruption of granules, and release of cellular constituents into the surrounding environment. Both cells are necessary for hemostasis but in addition, amoebocytes are necessary for the coagulation of blood. The presence of the entire coagulation system in amoebocytes provides the basis for suggesting that coagulation in animals was initially a cellularly based function. The role of the amoebocyte in controlling infection and its reaction to endotoxin suggest that the response of platelets and the blood coagulation system in various mammals to gram-negative infection or endotoxin is a remnant of this mechanism.