# FREE COMMUNICATIONS
# 12.45 – 14.00

## Prothrombin Activation

Queen Elizabeth Hall

*Time*

*12.45* **0768** PROTEIN CONCENTRATION DEPENDENCE OF CALCIUM BINDING TO PROTHROMBIN FRAGMENT-1

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Calcium binds to prothrombin and prothrombin fragment-1 with apparent positive cooperativity. It has previously been suggested that the cooperativity is induced by a conformation change in the protein. Another process which can induce cooperativity is ligand induced self-association. Calcium has been shown to induce dimerization in prothrombin and fragment-1. If such an intermolecular process is responsible for the apparent cooperativity, the calcium binding should become non-cooperative at the limit of zero protein concentration. The protein concentration dependence of calcium binding to fragment-1 was investigated using ultraviolet difference spectroscopy and the Hummel-Dryer equilibrium binding technique. A maximum of 10 sites was found on fragment-1 at high calcium concentration (10 mM). The maximum in the Scatchard plot was observed to decrease at fragment-1 concentrations less than 10 μM. The difference spectral data was analyzed using the equilibrium binding data. The apparent dissociation constant obtained from difference spectral titrations is protein concentration dependent. At low protein concentration (1-2 μM) the maximal spectral change occurs with 2 or less Ca ions bound, while at higher concentrations (50-100 μM), the maximum is reached after ~5 sites are filled. (Supported by HL14147, Specialized Center of Research on Thrombosis, NHLBI).

*13.00* **0769** IMMUNOCHEMICAL STUDIES OF BOVINE PLATELET FACTOR V.

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Utilizing new knowledge of the subunit structure of bovine plasma factor V, we studied factor V derived from bovine platelets. Platelets were freed of plasma factor V by gel filtration and solubilized in 0.2% Triton X-100. Incubation of this extract with a specific antiserum against purified plasma factor V rapidly inactivated platelet factor V. Antiserum absorbed with platelet extract failed to inactivate plasma factor V, further indicating that platelet factor V is antigenically related to plasma factor V. Platelet factor V in the extract increased 4.7 fold when activated with thrombin while factor V released from gel filtered platelets by collagen was activated 7.5 fold. In contrast, an 18 fold increase in activity was observed when diluted plasma or purified plasma V was incubated with thrombin suggesting that platelet factor V is partially activated during release. On immunoelectrophoresis, factor V released by collagen or extracted with Triton appeared as a single line close to the origin in contrast to plasma factor V which migrated toward the anode. Incubation of the collagen releasate with antiserum to plasma factor V resulted in an immunoprecipitate which when electrophoresed in SDS gave a single band with $M_r$=270,000, corresponding closely to the h chain of bovine plasma V, $M_r$=290,000. The alteration in platelet factor V during release may facilitate its role in prothrombin conversion.