Time
13.15 **0770** INTERACTION OF FACTOR V AND FACTOR Va WITH PLATELETS

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We have used homogeneous single chain bovine factor V to examine the binding of both factor V and factor Va to bovine platelets, as well as to develop a double-antibody radioimmunoassay (RIA) to measure intrinsic platelet factor V. Reaction of the protein with ^{125}I Bolton-Hunter reagent produced a labelled product which retained 90% of its cofactor activity and gave products indistinguishable from native factor V following thrombin activation. When incubated separately with washed bovine platelets, both ^{125}I-factor V and Va underwent saturable and exchangeable binding. There are high affinity binding sites to which 500-900 V(Va) molecules are bound per platelet with an apparent dissociation constant of 3×10^{-10}M, as well as binding sites of slightly lower affinity ($K_d = 3 \times 10^{-9}$M) to which as many as 3500 V (Va) molecules are bound per platelet. Thrombin pretreatment of the platelets was not required for the binding of either factor V or Va. The RIA data for Triton X-100 lysed, washed bovine platelets revealed that 400-1000 intrinsic factor V molecules were present per platelet. Factor V clotting assays produced results consistent with the RIA data. These studies suggest that the factor V molecules intrinsic to the platelet are equivalent to the number of high affinity factor V (Va) binding sites present on the platelet membrane surface. (Supported by Grant HL-17430 and the Mayo Foundation).

13.30 **0771** THE ACTIVATION OF PROTHROMBIN BY X_{1A} & X_{2A} AND INHIBITION BY FRAGMENT 1

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The rate of conversion of prothrombin by Xa and Ca^{++} is increased 20,000-fold following the addition of Factor V and phospholipid. A comparison of the rates of thrombin formation obtained with X_{1A} and X_{2A} showed that X_{1A} had only 70% the activity of X_{2A}. The addition of Fragment 1 (2.4nmoles) to mixtures of Xa, prothrombin (4.3nmoles), phospholipid and Ca^{++} reduced the rate of thrombin formation by 40%. This inhibition was relieved by adding an optimal amount of Factor V. When des-carboxy Fragment 1 was added no inhibition in the rate of thrombin formation was observed. These results suggest that the inhibition caused by Fragment 1 is dependent on intact γ-carboxy-glutamic acid residues and is due to the competition for the Ca^{++} mediated prothrombin binding sites on the phospholipid or Xa. The effect of Factor V in abolishing the inhibition caused by Fragment 1 indicates the importance of the interaction of prothrombin with Factor V in forming the prothrombinase complex.

13.45 **0772** FUNCTION OF PREVIOUSLY UNRECOGNIZED PLASMA PROTEIN M IN THROMBIN GENERATION

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Purified prethrombin 1 converts to thrombin in the presence of purified Ac-globulin, purified Factor Xa, phospholipid and calcium ions (Baker and Seegers, Thromb. Diath. Haemorrh. 17: 205-213 (1967)). The yields are, however, low. By adding a protein fraction isolated from bovine prothrombin complex the yield becomes maximum. This fraction we call Protein M. It is obtained by fractionating bovine prothrombin complex (McCoy and Seegers, Thromb. Res. 1: 461-472 (1972)) using a DEAE-Sephadex A-50 column 1.5 x 15 cm as previously described (Novoa, Seegers and Hassouna, Prep. Biochem. 6: 307-338 (1976)). Prothrombin and Factor IX are eluted with 0.25M sodium chloride followed by Protein M at 0.30M sodium chloride. Protein M is obtained as a single component from a Sephadex G-100 column (2.5 x 180 cm). Until now we have not been able to identify Protein M with any previously known coagulation factor. Protein M is a single chain protein with an apparent molecular weight near 60,000 as determined by polyacrylamide gel electrophoresis. Like prethrombin 1, highly purified bovine prothrombin also requires Protein M for maximum thrombin production in the presence of purified Ac-globulin, purified Factor Xa, phospholipid and calcium ions. In relatively large amounts Protein M shortened the prothrombin time of normal bovine plasma as well as plasma samples drawn during Coumadin administration.