Time

14.30 **0394** THE MECHANISM OF ACTIVATION OF HUMAN COAGULATION FACTOR X

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During the coagulation process factor X is converted to a serine protease, factor Xa. The present study concerns the molecular events which occur during the activation of human factor X by Russell's viper venom and by the purified proteins of the extrinsic and intrinsic activator. Conversion of factor X was detected by amidolytic assays and SDS/polyacrylamide-gel electrophoresis.

The results show that all activators convert factor X (MW 72,000) to an active form. In the presence of phospholipid the initially formed factor Xa (MW 54,000) complicates the further sequence of reactions by catalysing a) the conversion of factor Xa to a second active form (MW 50,000), b) the conversion of factor X to an inactive product (MW 59,000) by splitting off a peptide containing the active site serine, and c) the further degradation of the 50,000 and 59,000 components to a smaller component (MW 40,000).

Comparison of these data with those reported for bovine factor X suggests that the mechanism of activation of human factor X is more complicated. The inactivation of both factor Xa and factor X by product factor Xa might be considered as important regulatory principles.

14.45 **0395** STUDIES OF THE ACTIVATION OF HUMAN FACTOR X IN PLASMA.

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Purified human Factor X (FX) was used to develop a new assay and studies were made of the activation of FX initiated by tissue factor (TF) in normal and various deficient plasmas. FX was purified to > 95% homogeneity from commercial FIX concentrates using DEAE-Sephadex, heparin-agarose and preparative polyacrylamide gel electrophoresis. FX (59,000 MW), a glycoprotein, consists of two disulfide linked chains (42,000 and 17,000 MW). The carbohydrate of FX was labeled by a mild oxidation-reduction procedure using periodate and $NaB(^3H)_4$. The specific radioactivity was 1.58 μCi/unit FX with no loss of procoagulant activity. The amount of TCA-soluble ^3H-activation peptide released by RVV activation was directly correlated with both FX_a clotting and amidolytic (S-2222) activity. The assay background was much less than 1% of the total radioactivity and the maximum amount of ^3H-peptide released by RVV was 78% of the total radioactivity. In studies of the activation of ^3H-FX in normal and various deficient plasmas initiated by various dilutions of TF, there was a significant reduction of FX activation in FVII-, FIX-, and FVIII-deficient plasmas compared to normal or FXI-deficient plasmas. The potential influence of FIX and FVIII on the activation of FX in the presence of dilute TF emphasizes the need to revise current concepts of coagulation pathways and may be related to the abnormalities associated with Hemophilias A and B.

15.00 **0396** THE ACTIVATION OF BOVINE FACTOR X BY FACTORS IXa AND VIII.

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The esterase activity of bovine factor Xa on the synthetic substrate α-N-benzoyl-L-arginine ethyl ester can be used to follow the activation of factor X by the intrinsic pathway of coagulation. Bovine factor IXa activates factor X in a reaction having an absolute requirement for calcium ions, but little affected by phospholipid. At a molar ratio of factor IXa to factor X of 1:10, 3% of the factor X is activated after five minutes incubation at 37°C; the inclusion of bovine factor VIII in the incubation mixture results in 35% activation in the same period. Factor VIII cannot activate factor X in the absence of factor IXa. There is not an absolute requirement for phospholipid in the interaction of factors X, IXa and VIII, but the addition of crude cephalin further accelerates the rate of factor X activation. Hirudin does not affect the interaction of factors IXa and X in the absence of factor VIII, but does reduce the stimulatory effect of factor VIII on the rate of formation of factor Xa by a factor of between two and four. As hirudin specifically inhibits thrombin, this effect on factor X activation is likely to reflect the presence of trace amounts of thrombin in the incubation mixtures. The incomplete inhibition by hirudin of the effect of factor VIII on factor X activation suggests that 'activation' of factor VIII by thrombin cannot be a prerequisite for its interaction with factors IXa and X.