THE ROLE OF COMPLEMENT AND PLATELETS IN THE DISSOLUTION OF THROMBIN-INDUCED CLOTS. Fletcher B. Taylor, Jr. University of Oklahoma Health Sciences Center, Oklahoma City, OK.

The dilute whole blood clot lysis assay has been used in diagnosis of patients with deep vein thrombophlebitis and pulmonary embolism. Because of its clinical value it has also been the subject of biochemical and physiologic studies of clot lysis of normal diluted blood. This assay has shown that the behavior of platelets in that clot lysis as well as clot retraction are platelet dependent. Further, this contribution of platelets is temperature sensitive whereas the rate of fibrin clot formation is not. Thus, this assay offers a convenient model for functional and morphologic studies of temperature induced discontinuity of platelet-fibrin assembly and the interaction of platelet and fibrin in clot formation, retraction and lysis. In these studies the release of serotonin from platelets was correlated with clot formation, retraction, lysis, and clot morphology at 1) 37°, 4° and 4°-37° at 5, 15, 30 and 60 minute intervals. The results suggest that both platelets and fibrinogen are influenced by thrombin used in the assay and that under certain temperature conditions the platelets will release their contents out of phase with the assembly of the fibrin network. In cases where this discontinuity exists, the diluted clots will not retract or lyse normally.

ANTIPLASMIN, THE FAST-REACTING PLASMIN INHIBITOR OF HUMAN PLASMA. D. Collen and B. Wiman. Lab. of Blood Coagulation, Dept. of Medical Research, University of Leuven, Belgium and Dept. of Medical Chemistry, University of Umeå, Sweden.

Recently a new plasmin inhibitor was discovered in human plasma by three different groups (Eur. J. Biochem. 69, 209, 1976; J. Biol. Chem. 251, 5956, 1976; Biochem. J. 159, 545, 1976). This inhibitor is responsible for the fast-reacting plasmin-neutralizing activity of human plasma and forms a very stable 1:1 molar complex with plasmin, which is devoid of protease and esterase activity. The concentration of antiplasmin in human plasma is approximately 1 μ M (2/3 that of plasminogen).

We have purified the inhibitor by affinity chromatography on insolubilized plasminogen, followed by DEAE-Sephadex and concanavalin A chromatography. It is a glycoprotein with a mol. wt. of 67,000 (SDS-gel electrophoresis), a carbohydrate content of 14%, and amino terminal sequence Asn-Gln-Glu-Gly (Wiman and Collen, submitted).

The inhibitor interacts strongly with the light chain of plasmin (probably through reaction with the active site serine), and weakly with the heavy chain (probably through interaction with the lysine-binding site), suggesting that antiplasmin not only functions as an inhibitor of plasmin, but also interferes with the interaction between plasmin(ogen) and fibrin.

PURIFICATION AND SOME PROPERTIES OF TISSUE PLASMINOGEN ACTIVATOR. P. Wallén and M. Rånby. Department of Chemistry, Umeå University, Umeå, Sweden.

A highly purified preparation of plasminogen tissue activator from pig heart has been prepared for studies on its chemical properties and on its interaction with fibrin and fibrinogen from the functional point of view. Essential steps in the purification procedure are two affinity adsorptions involving two different sites in the activator molecule.

1. Affinity adsorption to fibrin followed by elution in two steps with potassium thiocyanate and phenylethylamine. 2. Affinity chromatography on Sepharose-arginine. The final product moves as a single band on SDS-polyacrylamide gel electrophoresis although an additional trace component is sometimes observed. The specific activity is at least 150 000 CTA units/mg protein as measured in a fibrinolytic test system. The molecular weight measured by SDS-polyacrylamide gel electrophoresis is 63 000 dalton before reduction. Reduced samples still appear as one fraction on electrophoresis, but the molecular weight has diminished to about 31 000 dalton, i. e. half the original value. The highly purified activator is labile in neutral solutions of low ionic strength but is stabilized by increasing the ionic strength to 1. The firm and specific adsorption of tissue activator to fibrin is presumably of physiological importance. Attempts are therefore made to study sites in fibrin (fibrinogen) with affinity for tissue activator. Preliminary experiments indicate that one site is situated in the part of A α chain which is released from fibrinogen or fibrin early during digestion with plasmin.