M. Zuzel, W. Irving and G. V. R. Born (Department of Pharmacology, University Medical School, Hills Road, Cambridge, England): **Membrane Transports of Platelets Present in Plasma During Clotting and Lysis.** (9)

During blood clotting and subsequent fibrinolysis a major proportion of platelets survive as single entities. Platelets exposed to thrombin *in vivo* or *in vitro* survive normally in the circulation (Reimers et al., 1973, *Brit. J. Haemat.*, *25*, 675), in spite of having undergone a release reaction.

Our experiments were to find out whether clotting and lysis of plasma impair transport functions of platelet membranes. Human citrated platelet -rich plasma was incubated at 37° with thrombin plus fibrinolytic activator. Rapid clotting was followed by complete fibrinolysis within 3 min. By then about 60% of radioactive 5-hydroxytryptamine previously taken up by the platelets was released; this was accompanied by the loss of about 40% of platelet potassium which was subsequently re-accumulated. The initial velocities of uptakes of 5-hydroxytryptamine and adenosine were almost identical in platelets before and after clotting and lysis. Therefore, at least three energy-requiring transport processes in the platelet membrane were unaffected by the chemical events associated with clotting and lysis of platelet-rich plasma.

M. Koch, B. Binder and W. Auerswald (Dept. of Physiology, School of Medicine, University of Vienna, 17 Schwarzspanierstr. A-1090 Vienna, Austria): **Release of Fibrinolytically Active Compounds During Platelet-UK Interaction.** (10)

In view of contradictory reports concerning the influence of platelets on the fibrinolytic activity in vivo and in vitro, we studied the effects of washed platelets on the fibrinolytic activity of added urokinase (UK). Different amounts of UK (0.6 to 2.5 CTA units per ml, purified UK-preparation) were mixed with washed platelets numbering 1,000 to 1,000,000,000 per ml. The fibrinolytic activities of these mixtures as well as of the platelet-free supernatants there of were tested on native and heated fibrin plates. Neither UK nor platelets alone nor the mixtures of both revealed any fibrinolytic activity on heated fibrin plates. In addition, no plasminogen activator activity of the native platelets could be detected.

The presence of more than 10,000,000 platelets per unit UK suppressed the kinases's activity progressively as the platelet number increased. With less than 10.000.000 platelets per unit UK the kinase activity was augmented to a maximum when the platelet count reached about 200,000 per unit UK. The supernatants of the platelet-UK mixtures reflected the same activities as the mixtures. Using plasmin instead of UK in the mixtures with platelets similar results were obtained. In control experiments incubating platelets with albumin- or buffer-solutions the supernatants were without effects. Furthermore at no of the mentioned concentrations of UK added to platelets were any effects found on their count, their adherence to glass, and their spreading ability.

In preliminary investigations supernatants from platelet-UK mixtures were gel fractionated on Sephadex G-150, to determine the presence of fractions with inhibiting and/or potentiating effects on fibrinolysis. An inhibiting fraction of low mol. wt. and probably two potentiators of higher mol. wt. have thus far been found.

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U. Okamoto and J. Yamamoto (Kobe-Gakuin University. Tarumi. Kobe. Japan): **A Smaller Molecule SK-Reactive Protein (Plasminogen-Proactivator) Derived from the Macromolecule of Human Plasma Fraction I.** (11)

The SK-reactive plasminogen-proactivator reported in detail by previous workers assumed a macromolecule protein though still impure. A smaller molecule proactivator highly purified is described in the present paper.

Fraction I of human plasma was chosen as staring material after preliminary studies.

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The macromolecule obtained by gel filtration had low specific activity of SK-reactive protein (proactivator) but little UK-reactivity, showing constantly a large molecular weight by repeating gel filtration. The successful purification of proactivator was achieved by Lysine-Sepharose affinity chromatography, using as eluant t-AMCHA (fibrinolysis inhibitor) which was found suitable.

The proactivator fraction thus obtained showed the high sensitivity to SK, other proteins being removed satisfactorily and the specific activity being increased extremely. Even a trace of plasminogen was not detected. Molecular weight of the proactivator was found 98,000 by gel filtration.

The results indicate that the smaller molecule proactivator described is a basic entity of SK-reactive proactivator of plasminogen.

G. P. M. Crawford, D. Ogston and A. S. Douglas (Department of Medicine, University Medical Buildings, Foresterhill, Aberdeen, Scotland): **The Contribution of Individual Protease Inhibitors to Plasma Antiplasmin Activity.** (12)

The plasma proteins alpha-1-antitrypsin, alpha-2-macroglobulin, antithrombin III, Cl inhibitor and inter-alpha trypsin inhibitor have been shown to be capable of inhibiting plasmin activity. Whole plasma possesses both 'rapid' and 'progressive' plasminneutralising activity: the study was intended to assess the contribution of individual protease inhibitors to this plasminneutralising activity.

The antiplasmin properties, assessed as 'rapid' and 'progressive' by the neutralisation of plasmin in a caseinolytic assay, and the levels of the individual protease inhibitors, assayed by single radial immunodiffusion, were measured in the plasma of 35 subjects. Statistical analysis using zero order correlation coefficients and zero order partial correlation coefficients indicated that 'rapid' and 'progressive' antiplasmin activity correlated with the plasma concentration of alpha-2-macroglobulin and alpha-1-antitrypsin respectively, but not with antithrombin III, Cl inhibitor and interalpha trypsin inhibitor.

It is concluded that alpha-2-macroglobulin and alpha-1-antitrypsin are the major plasma inhibitors of plasmin and that immunological determination of these proteins provides a valid assessment of plasma antiplasmin activity.

S. Oshiba and T. Ariga (Dept. of Physiology, Nihon Univ. School of Med., Tokyo, Japan): **Purification and Characterization of Bilokinase, a Biliary Plasminogen Activator.** (13)

A biliary plasminogen activator was first found in human and animal bile by Oshiba et al. and termed bilokinase (Jap. J. Physiol. *19*, 212, 1969). Purification of this activator from bovine bile was later achieved through a six-step procedure consisting of a new extraction method using $(NH_4)_2SO_4$/acetone under controlled condition, followed by chromatography with SP-, G-100 and CM-Sephadex. The highly purified preparation exhibited a single band in disc electrophoresis. A sedimentation velocity study gave $S_{20,w}$ value of 3.32 S. The molecular weight was estimated at 58,000 from the elution volume/void volume ratio. A comparative study with urokinase showed a different mode of action for its substrate specificity toward AGLMe (K_m) and its behavior toward (AMCHA (K_i). Amino acid analysis of our preparation indicated a higher lysine but lower tyrosine content than in human urokinase (W. F. White et al., Biochem. *5*, 2160, 1966), among some other differences. A distinct immunological dissimilarity between bilokinase and urokinase was eluciadated by Ouchterlony's method and from inhibition with anti-bilokinase serum.

P. Ostendorf and K. N. von Kaulla (University of Colorado Medical Center, Denver/ Colorado, U.S.A.): **Endogenously Increased Fibrinolytic Activity Induced by Liver-Bypass in Pigs and its in Vitro Synergism with Synthetic Fibrionolytic Compounds.** (14)

The marked increase of fibrinolytic activity during the unhepatic phase during liver transplantation in animals and man is well known. Based on these observations the liver-bypass in the pig was used for producing endogenous increase of fibrinolytic activity of