C. S. P. Jenkins, D. R. Phillips, K. J. Clemetson, Dominique Meyer and Marie-José Larrieu (Institut de Pathologie Cellulaire, Hôpital de Bicêtre, 94270 Le Kremlin Bicêtre, France and Theodor Kocher Institute, Bern 9, Switzerland): **Platelet Membrane Glycoproteins Implicated in Ristocetin-Induced Aggregation. (453)**

Ristocetin induces aggregation of platelets in the presence of Willebrand Factor. Certain preparations of ristocetin when preincubated with washed normal platelets render the platelets less responsive to the addition of Willebrand Factor. It has been demonstrated that such preparations are contaminated with a proteolytic enzyme(s). Platelets treated in this way with different ristocetin preparations were analysed using disc gel electrophoresis. Membrane glycoproteins I and II were depleted only when using contaminated ristocetin preparations. Platelets labelled with ^{3}H-adenine when treated with this ristocetin did not demonstrate leakage of ^{3}H-labelled material, thus showing that the platelet membrane remained intact.

Platelets were treated with proteolytic enzymes of known specificities and following further washing were tested in an aggregating system consisting of ristocetin and Willebrand Factor. The aggregation response was markedly reduced compared to control platelets. These results point to the glycoproteins of the platelet membrane being part of the receptor for the ristocetin-Willebrand Factor complex.

G. Gasic, T. Gasic, B. Hsu, P. Koch and S. Niewiarowski (Dept. Path., Univ. Penna Sch. Med. and Spec. Ctre Thrombos Res and Dept. of Med., Temple Univ. Hlth. Sci Ctre, Philadelphia, Pa.): **Platelet Interaction with Tumor Cells. (454)**

Previous investigations demonstrated that mouse tumors cause platelet aggregation (PA) and increase platelet turnover. Depletion of platelets by neuraminidase and inhibition of PA by aspirin reduced the number of metastases (Gasic et al., *Int. J. Cancer 11*, 704, 1973). The purpose of this investigation was to study further interaction of cells from various mouse and human tumors with platelets. Cells of 7 mouse tumors (1 mammary adenocarcinomas, 5 sarcomas, 1 melanoma) and 14 human tumors (8 breast, 3 colonic adenocarcinomas, 1 cancer of the ureter, 1 Wilms tumor, and 1 neuroblastoma) aggregated homologous platelets suspended in heparinized plasma. Three mouse tumors (2 mammary and 1 sarcoma) and 5 human tumors (2 breast, 1 sarcoma, 1 Wilms, and 1 neuroblastoma) did not. PA was accompanied by the release of radio-activity from ^{14}C-serotonin labeled platelets (range 15–90%). PA activity was not correlated with fibrinolytic or procoagulant activity. The contribution of plasminogen activators, thrombin, and tumor immune complexes has been excluded. However, gamma globulin of tumor bearing mice contained a "blocking factor" which delayed PA. Since enzymatic removal of ADP reduced PA it is possible that the ADP release either by tumors or by platelets played a contributory role. The pattern of PA by tumor cells ressembled that induced by collagen. Indeed preliminary evidence suggests that collagen-like material associated with tumor cells might be involved in platelet adherence to these cells and subsequent aggregation.

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M. Steinbuch, A. Friess, J. Drouet and P. Amouch (Centre National de Transfusion Sanguine, 6, rue Alexandre-Cabanel, Paris XVè): **Absence of Interaction between Streptokinase/Plasmin(ogen) Complexes and α_2-Macroglobulin (α_2M). (455)**

Streptokinase, plasmin(ogen), the activator complex and α_2M have different, well defined electrophoretic mobilities in agarose/acrylamide in function of their electric charge and molecular size. Highly purified I^{131}-labeled streptokinase has been used to study whether an interaction with α_2M can be seen. No radioactivity associated with α_2M could be found. Thus, with respect to α_2 M, streptokinase does not behave like urokinase. Furthermore, activator complexes formed in the cold (4° C) to avoid any dissociation did not react with α_2M either as shown by the same technique whereas I^{131}-labeled plasmin became normally fixed to α_2M under the same conditions. Several anodic radioactive

bands are observed after the interaction between I^{131}-labeled streptokinase and plasmin(ogen) thus showing a striking modification of the streptokinase molecule.

It can be concluded that the activator complex is not inhibited by α_2M.

Peter C. Harpel (The New York Hosital-Cornell Medical Center, N.Y. N.Y., U.S.A.): **Relative Affinity of Plasmin for the α_2-Macroglobulin, C1 Inactivator and α_1-Antitrypsin Inhibitors.** **(456)**

The quantitative contribution of three major plasma protease inhibitors in binding plasmin has been studied. Mixtures of plasmin and each of the purified inhibitors were analyzed by SDS-acrylamide gel electrophoresis. Plasmin remained bound to its inhibitors in the presence of SDS and urea. A 1 : 1 molar ratio for complex formation was established, and treatment of the complexes with a disulfide bond reducing agent showed that the light chain of plasmin contained the binding sites for both $C\bar{1}$ inactivator and α_1-antitrypsin. Limited degradation of all three inhibitors by plasmin was observed, and the altered inhibitor remained complexed to the enzyme. The competitive binding of ^{125}I plasmin to mixtures of these inhibitors was followed by sucrose density ultracentrifugation and by SDS-gel electrophoresis. In mixtures containing physiologic molar ratios of enzyme and inhibitors, over 80% of the bound plasmin was complexed to the α_2-macroglobulin (α_2M). No evidence for an exchange of plasmin between the inhibitors was obtained.

D. Collen (Laboratory of Blood Coagulation, Department of Medical Research, University of Leuven, Belgium): **Quantitative Estimation of Thrombin-Antithrombin III and Plasmin-Alpha$_1$-Antiplasmin Complexes in Human Plasma.** **(457)**

During activation of the coagulation or fibrinolytic system in human plasma neoantigens evolve, associated with the thrombin-antithrombin III (T-AT) and plasmin-α_1-antiplasmin (P-AP) complexes (D. Collen and F. De Cock, Thromb. Res., *5*, 777, 1974). A tanned red cell hemagglutination inhibition immunoassay (TRCHII) has been adapted to the quantitation of these complexes in plasma. Experiments with partially absorbed antisera revealed that fresh plasma had a titer of 4 to 32 in the TRCHII for T-AT and of 4 to 16 in the TRCHII for P-AP. Serum reacted 8 to 16 times better than plasma in the TRCHII for T-AT and urokinase activated plasma 32 to 128 times better than plasma in the TRCHII for P-AP.

During streptokinase (SK) therapy in 3 patients (infusion of 600,000 IU Kabikinase® over 30 min) a marked increase in the P-AP complex titer, approaching or equalling that of the subject's plasma activated in vitro with urokinase, was observed at the end of the SK infusion. The titer remained high during the first three hours and was still higher than the pre-infusion value after 24 hours, suggesting that this complex has a half-life of several hours in vivo. During reptilase therapy in 3 patients (infusion of 2 ml Defibrase® over 1 hour) the P-AP complex titer increased gradually but submaximally over a period of several hours and remained elevated for at least 48 hours, indicating that the plasminogen consumption observed during reptilase therapy is due, at least in part, to a secondary fibrinolytic response.

Further studies to show whether these assays can be converted to rapid, simple and specific tests for the diagnosis of low grade or regional in vivo coagulation or fibrinolysis in clinical conditions are in progress.

E. A. van Royen, W. Kempes-van Leeuwen, M. Vos and K. W. Pondman (Dept. fo Blood Coag. Res., Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam): **Cold Promoted Activation of Factor VII and $C\bar{1}$ Esterase Inhibitor.** **(458)**

The cold promoted activation (CPA) of factor VII involves the conversion of prekallikrein into kallikrein by factor XII (Gjønnaess et al.). Since $C\bar{1}$ esterase inhibitor ($C\bar{1}$INH) and macroglobulin block the prekallikrein activating activity of factor XII, the role of $C\bar{1}^2$INH in CPA was investigated especially because $C\bar{1}$INH deficient plasma was available. Purified $C\bar{1}$INH was an effective inhibitor of CPA in final concentrations of 10–100 U/ml, only if