these levels reached pre-aggregation values again. Since the levels of other glycolytic intermediates were only slightly changed, the extra flux may be related to the increased levels of intermediates immediately formed after the phosphofructokinase reaction, e.g. to increased phosphofructokinase activity.

Furthermore since aggregation is maximal 2 min after collagen addition the prolonged increase in phosphofructokinase activity suggests that glycolysis mainly has the task to replenish energy utilized by the aggregation and release process.

P. Kubisz and J. Suranová (Department Hospital, 02201 Čadca, Czechoslovakia): **Mechanism of Platelet Dependent Retraction of Reptilase Clots. (516)**

Effects of agents affecting cyclic AMP and microtubule function.

Human platelets stimulated by ADP or collagen caused retraction of fibrin formed in the presence of highly purified Bothrops atrox thrombin like enzyme (Reptilase®).

Incubation of normal human platelets with compounds increasing cyclic AMP levels in the cells or with exogenous db-cyclic AMP, reduced considerably or almost completely (depending on the concentration and incubation time) the retraction of ADP-, or collagen – reptilase clots. This type of retraction requires the presence of Ca^{2+} – ions and is strongly influenced by the agents which directly affect the platelet microtubules.

The data suggest that this manifestation of platelet contractile activity depend upon functional state of microtubular structures and may be modulated by the platelet cyclic AMP/Ca^{2+} ratio.

J. Over, I. Bakker-Woudenberg, B. N. Bouma, J. A. van Mourik and J. J. Sixma* (Dept. of Haematology, University Hospital, Utrecht and *Lab. of Biochemistry, Pediatric Clinic, Binnengasthuis, Amsterdam, The Netherlands): **Partial Purification and Characterization of Factor VIII-Activity Present in the Supernatant of Cryoprecipitates. (517)**

A relative higher percentage of the initial factor VIII activity is found in the supernatant of plasma from patients with von Willebrand's Disease than in that from normals after cryoprecipitation. Moreover no factor VIII related antigen can be demonstrated in the supernatant after cryoprecipitate. A further study of the characteristics of this cryosupernatant-factor VIII seemed therefore of interest.

Cryosupernatant-factor VIII was partially purified by polyethyleneglycol and ammoniumsulfate precipitation, followed by Sepharose 6B gelfiltration. Cryosupernatant-factor VIII was eluted at the void volume of Sepharose 6B columns, but in contrast to cryoprecipitated factor VIII it was retarded on Sepharose 2B columns.

Cryosupernatant-factor VIII has antigenic determinant(s) in common with normal factor VIII.

Von Willebrand plasma's obtained 6 to 40 hours after transfusion with normal cryoprecipitate were applied to gelchromatography on Sepharose 6B columns. The factor VIII activity was eluted at the void volume. On Sepharose 2B the elution pattern of the factor VIII activity was comparable to that of normal plasma.

Prudence Bird and C. R. Rizza (Oxford Haemophilia Centre, Churchill Hospital, Oxford): **Coagulation in an Agarose Gel and its Application to Detecting Factor VIII Clotting Activity Associated with Factor VIII-Related Antigen. (518)**

The coagulation of citrated plasma in an agarose gel treated with thrombin or calcium chloride may be detected by a change in the gel opacity. Experimental evidence suggests that this opacity represents fibrin formed within the gel matrix. The coagulation of citrated 0% factor VIII haemophilic plasma in an agarose gel treated with calcium chloride is delayed compared with normal plasma. A method for detecting factor VIII clotting activity in agarose has been developed based on factor VIII promoting the coagulation of haemophilic plasma in agarose.

Using this test to detect factor VIII clotting activity in a one dimensional Laurell electroimmunoassay for factor VIII-related antigen, all factor VIII clotting activity detected is found in the same position as the factor VIII-related antigen immunoprecipitate. This result suggests that the molecule containing factor VIII clotting activity carries factor VIII-related antigen determinants.

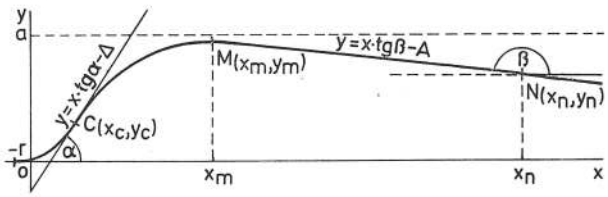
R. Pieptea, D. Pieptea and M. Pieptea ("V. Babes" – Hospital, Bucharest, Romania): **Mathematical Study of the Blood Coagulation Phenomenon. (518 bis)**

Mathematical analysis of the coagulation process by means of thrombodynamogram made it possible to establish some new and important graphical elements (2) and the analytical expressions (1) of this phenomenon (F[x]):

(1)

$$F(x) = \begin{cases} 0 & x \in (-r, o) \\ \dfrac{ax^2}{x^2 + \lambda} + E(x) & x \in (o, x_m) \\ x.tg\beta + A & x > x_m \end{cases}$$

(2)



where a = quantic coagulation parameter ($a \simeq y_m$); λ = coagulation rhythm parameter ($\lambda = 3 \times \frac{2}{c}$); $C_{(x_c, y_c)}$ = thrombodynamic center (inflection point of the curve); $M_{(x_m, y_m)}$ = point of maximum amplitude of the curve; $N_{(x_n, y_n)}$ = arbitrary point on the descending slope. Correct identification and interpretation of these elements raises the expressivity of the thrombodynamogram for evaluating coagulation phenomena.

F. M. Booyse, S. Bell, B. Sedlak and M. E. Rafelson Jr. (Dept. of Biochemistry, Rush University, Chicago, IL 60612, U.S.A.): **Development of an in Vitro Vessel Wall Model for Studying Certain Aspects of Platelet-Endothelial Interactions. (518ter)**

Cultured bovine aortic endothelial cells were characterized by the presence of Weibel-Palade bodies, pinocytotic vesicles, Factor VIII antigen and thrombostenin-like contractile proteins. Longitudinal sections of cells showed the presence of an extensive matrix of 100–110 Å extracellular filaments. Treatment of monolayers of these cells with serotonin, thrombin, trypsin, endotoxin and heat caused contraction and exposure of the 100–110 Å filaments. Cultured cells were grown on plastic cover slips (9×35 mm, $3.5–4 \times 10^5$ cells). Post confluent cultures (2 weeks) were treated (activated) with one of the above agents and exposed to platelets, laleled with ^{125}I (lactoperoxidase method) or ^{14}C-amino acids, for various periods of time. Cover slips were washed and counted. Initial platelet-endothelial interactions were linear and could be expressed quantitatively by plotting radioactivity (CPM or calculated number of platelets) per $3.5–4 \times 10^5$ endothelial cells vs. exposure time. Non-activated control values were subtracted in each case. PGE_1, cAMP, ATP and aspirin inhibit this interaction. Citrate and EDTA decreased the interaction. Interaction was increased by red blood cells and rotational flow rates and unaffected by plasma proteins. Preliminary electron microscopic observations suggest platelet attachment at the sites of 100–110 Å microfilament abundance. The feasibility of developing a quantitative *in vitro* model for studying platelet-endothelial interactions under defined experimental conditions will be considered further.

P. Hilgard (Universitätsklinikum (Tumorforschung), 43 Essen, Germany): **Predisposition to Thrombosis in Experimental Cancer. (519)**

In accordance with clinical findings in malignant diseases, the growth of allogeneic and autochthonous rat tumours induced characteristic changes of the clotting mechanism of