reduction of both biological and immunological AT-III activity was found in the samples defibrinogenated by heating (10 min), as compared with the other 3 procedures. These results indicate that Reptilase-R and Boropase-R are suitable reagents for defibrinogenation of plasma prior to AT-III assay; heat precipitation can also be used, provided the duration of the incubation period is accurately controlled.

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H. E. Karges, N. Heimburger and C. Loechelt (Behringwerke D 355 Marburg/Lahn, BRD): **A Method for the Quantitative Determination of the Antithrombin III (AT III) a Citivity in Plasma and Serum Using the Complex Formation with Heparin. (100)**

A prerequisite of most AT III assays is the defibrination of the samples. Defibrination of plasma, however, is generally associated with a loss of AT III. This especially true of heat defibrination. Furthermore, according to our experience, most of the functional determinations don't correlate well with the immunological ones, and don't provide reproducible results. Therefore, it was the aim of our investigations to establish a method which omits the defibrination and yields reproducible results.

The method reported is based on the identity of AT III and heparin cofactor antithrombin II (AT II) respectively. AT III is converted by heparin from a progressive into an immediate inhibitor, allowing a plasma dilution of 1:50. Due to this high dilution, the defibrination step can be omitted and AT III determined in native plasma. To transform AT III completely into its heparin complex, 5 USP units heparin are used. α_2-macroglobulin up to 400% of normal plasma concentration does not influence the assay. When determinations are performed at a certain pH value, good reproducibility is obtained. The mean error of determinations of the same sample does not exeed 4%. Maximal deviations were in the range of 5 to 10%. The results of functional determinations are compared with those obtained by two immunological methods. Deviations scarcely exeed the limits of methodical errors.

N. O. Osamo (Haematology Unit, University of Benin, Benin City, Nigeria): **The Effect of Divalent Cations on the Interaction between Thrombin and Antithrombin. (101)**

The importance of calcium irons for the action of pro-coagulant blood clotting factors has been known since the time of Morawitz's classical theory and the practical application of this knowledge is demonstrated in the use of anticoagulants for the collection of blood used in laboratory investigations. The necessity for cations in the interaction between the pro-coagulant factors and their inhibitors has not been described. We now find that during the isolation and purification of antithrombin from normal plasma, the use of dialysis tended to diminish or even abolish the activity of this inhibitor. However, when the dialysate was reintroduced into the dialysed plasma or fraction containing the antithrombin, the activity was restored, although the dialysate itself showed no antithrombin activity. On reintroduction of divalent cations in place of the dialysate, the activity was also restored. It seems therefore, that these divalent cations are necessary for the interaction of thrombin and antithrombin. Although the exact mechanism of action of these cations has not been completely elucidated, it would appear that they act possibly by the removal of charges or by facilitation of bonding between the two reactants.

A. Hijikata, S. Okamoto*, K. Ikezawa*, R. Kikumoto**, S. Tonomura** and Y. Tamao*** (* Kobe University School of Med., Kobe; ** Central Lab. Mitsubishi Chem. Ind. Ltd., Tokyo, Japan): **Animal Experiments of a New Synthetic Thrombin-Inhibitor, Dansyl-Arginine-Methyl-Piperidine Amide. (102)**

A noticeable effect of dansyl-arginine-methyl-piperidine amide in animal experiments is described, which is one of the representative compounds of a new series of potent thrombin-inhibitors synthetized by the authors very recently. The inhibitor solution was intravenously administered to rabbits by means of the infusion pump to maintain the inhibitor in the circulatory blood at a concentration intended. When bovine thrombin was

intravenously administered (5 NIH units/Kg/min) for 75 minutes, a remarkable decrease of plasma fibrinogen content was observed in control, while any decrease of fibrinogen content was hardly observed at the inhibitor concentration of 10 ~ 20 μMin plasma. Further, the decrease of plateletes in number due to thrombin infusion (7.5 ~ 9 NIH units/Kg/min) for 5 minutes was suppressed satisfactorily at the inhibitor concentration of 1 ~ 2 μM in plasma.

These results indicate that the new synthetic thrombin-inhibitor is effective enough at a very low concentration to control the hazard due to thrombin given in vivo, encouraging the authors to extend the studies further toward the application.

D. Heinrich, C. Kessler, U. Stephinger, W. Kunkel and C. Mueller-Eckhardt (Department of Internal Medicine and Institute of Clinical Immunology and Blood Transfusion, D 6300 Giessen, Germany): **Action of HL-A Specific Antibodies on Platelets in Vitro. (103)**

Refractoriness to platelet transfusion in thrombocytopenic patients often is due to HL-A isoimmunisation. HL-A antibody-induced platelet alteration in vivo is associated with a reduction of platelet survival.

Action of HL-A specific antibodies on platelets in vitro was studied applying serological (absorption/elution-experiments; micro-complement fixation), morphological (platelet spreading, electron microscopy) and functional parameters (clot retraction, platelet agglutination, ^{14}C-serotonin uptake and ^{14}C-serotonin release).

Results: Incubation of platelets (PRP) with HL-A specific antisera induced a specific platelet agglutination with degranulation and ^{14}C-serotonin release, furthermore a specific inhibition of ^{14}C-serotonin uptake and clot retraction. Experiments with Prostaglandin E_1, Apyrase, Heparin, ASA and EDTA in connection with a HL-A specific LDH-liberation from platelets suggest a lytic, complement-dependent action of HL-A antibodies on platelets in plasma (PRP).

Experiments with washed platelets and HL-A specific eluates (D. Heinrich et al.: Vox Sang. *27*, 310, 1974) indicate a second, nonlytic action of HL-A antibodies on platelets. Cofactors of the latter reaction are Ca^{++}, fibrinogen and thrombin.

M. A. Chernesky and R. P. B. Larke (McMaster University, Hamilton, Canada): **Inhibition of Virus-Induced Platelet Aggregation by Treatment of Platelets with Neuraminidase. (104)**

Washed suspensions of rabbit or human platelets aggregate and release their constituents (adenine nucleotides, serotonin and lactic dehydrogenase) when mixed with Newcastle disease virus (NDV) or Sendai virus (paramyxoviruses) in a stirred system at 37° C. Treatment of rabbit platelets with chromatographically purified neuraminidase which removed 10–20 ug of sialic acid/10_1 platelets reduced, by more than 95%, platelet aggregation and release of constituents by virus; treated platelets were as responsive to stimulation with ADP or thrombin as were control platelets. Virus, which normally becomes associated with aggregated platelets, failed to attach to neuraminidase-treated platelets and was recovered in platelet supernatant fluids. Subsequent aggregation of these mixtures of virus and neuraminidase-treated platelets with thrombin failed to trap NDV in platelet aggregates. Electron microscopy (carbon replica and thin-sections) revealed an absence of virus attachment on surfaces of platelets treated with neuraminidase.

These data indicate that a receptor substance containing sialic acid present on the platelet surface is necessary to enable paramyxoviruses to attach and induce platelet aggregation and release of platelet constituents.

S. W. Jamieson (St. Mary's Hospital, London W. 2, England): **Modification of the Hyperacute Reaction in the Rat by Sulphinpyrazone. (105)**

It has been found that guinea-pig hearts are rejected in hyperacute fashion by the rat. By using inbred strains this model has been found to be markedly reproducible. Electron microscopic studies show that the platelet plays a critical role in the rejection process. An