H. Beeser, J. Fischer and R Merten (Dept. Exp. Hematology and Blood Transfusion, D 53 Bonn, Inst. Stand. u. Dokument., D 4 Düsseldorf, Fed. Republic Germany): **Progress in Proficiency Testing of the One-Stage Prothrombin time in the Federal Republic of Germany.** **(147)**

With regard to the small range of the therapeutic prothrombin complex levels the treatment with oral anticoagulants needs reliable laboratory controls. The results of the one-stage prothrombin time generally used as control method are however known to be poorly comparable between different laboratories. This difficulty arises particularly from the use of numerous types of thromboplastins, insufficient reference materials and calibration curves.

In the Federal Republic of Germany we three times a year conduct quality control trials of the one-stage prothrombin time according to the Bundesärztekammer (BAK) directions on external quality control of clinical laboratory methods. Participating laboratories are asked to determine prothrombin times of normal and abnormal freeze dried plasma samples using their own thromboplastin reagents and methods. For reasons remarked above a uniform evaluation of the results of all laboratories is impossible.

We can state: 1. Wide variation of the seconds for prothrombin times, even if identical thromboplastins are used. But the increasing use of mechanical devices brings along an obvious improvement. 2. The variability of the percentage results tends to decrease probably due to better calibration of the methods. 3. Prothrombin time ratios show a good comparability for each type of thromboplastin. Grouping of qualitively similiar thromboplastins has proved to be practicable. 4. There is found an increasing conformity of all mentioned parameters between expert labs and other labs.

K. Watanabe, F. C. Chao and J. L. Tullis (Department of Medicine, New England Deaconess Hospital, and Center for Blood Research, Boston, Massachusetts, U.S.A.): **Antithrombin Activity of Intact Human Platelets.** **(148)**

Antithrombin activity was identified in intact washed human platelets. Red cells and white cells had no antithrombin activity. There was no significant loss of platelet antithrombin activity after ten washings or after treatment with antibodies to antithrombin III or α_2-macroglobulin. Platelets from an afibrinogenemic patient showed almost the same antithrombin activity as normal platelets. Platelet antithrombin reacted with thrombin in less than 3 seconds. This rapid reaction was different from that of antithrombin III or fibrinogen. The clotting activity of ancrod was inhibited by fibrinogen but not platelets. Unlike plasma antithrombin III or fibrinogen, brief exposure to 60° C reduced platelet antithrombin activity. These results suggest that platelets possess a specific antithrombin with characteristics different from other known antithrombins.

S. Okamoto, A. Hijikata*, R. Kikumoto**, S. Tonomura** and Y. Tamao*** (* Kobe University School of Med. Kobe; ** Central Lab. Mitsubishi Chem. Ind. Ltd. Tokyo, Japan): **A Series of Extremely Potent Thrombin-Inhibitors Newly Synthetized.** **(149)**

Starting with the simple arginine derivatives having weak thrombin-inhibitory activity, ca. 320 chemical compounds were designed and synthetized by the authors, in such a way as increasing the inhibitory activity by stepwise chemical modifications. The extremely potent inhibitors thus obtained are described.

These inhibitors are chemcially naphthalene-sulfonyl-arginine derivatives, the basic structure being composed of three functional sub-units and designed to reflect the part of fibrinogen peptide to be split by thrombin.

One of the representatives, dansyl-arginine-ethyl-piperidine amide, inhibited 50% of thrombin activity at 0.1 μM when 5 μM fibrinogen was substrate. Similar result was also obtained when benzoyl-Phe-Val-Arg-pNA was substrate. The mode of inhibition was found competitive.

The inhibitors suppressed the platelets aggregation due to thrombin satisfactorily. However, the inhibition on plasmin, reptilase or trypsin was far weaker, indicating the

very high selectivity to thrombin. Their relatively low toxicity led the authors to extend the studies toward the animal experiments and results obtained will be reported elsewhere.

E. Marciniak (University of Kentucky Medical Center Lexington, Kentucky 40506 U.S.A.):
Adverse Effect of Heparin on Thrombin Inactivation. **(150)**

In the presence of heparin thrombin, although fast inactivated, impairs the inhibitory capacity of antithrombin III, in result of which the final amount of neutralized enzyme markedly decreases. This adverse effect of heparin was found during the reaction of purified thrombin with both purified human antithrombin III and native plasma hepariniz purified thrombin with both purified human antithrombin III and native plasma heparinized in vitro or in vivo. In the absence of heparin, at concentration equal to that in normal plasma antithrombin III binds 450 lowa units of thrombin; in the presence of heparin (at 1 unit concentration) this binding is reduced to 145 thrombin units. A fast depletion of inhibitory capacity is also evident after a stepwise addition of thrombin in small installments into a medium containing antithrombin III and heparin. Portions of enzyme initially added disappear with great velocity; subsequent additions, however, accumulate building up a high thrombin level not seen in the absence of heparin. The escalation of thrombin is reversely proportional to the reacting antithrombin III level, thus especially noticeable in antithrombin III deficient plasma. Residual thrombin left in the presence of heparin disappears at a fast rate upon a new addition of antithrombin III. No decrease in anticoagulant properties of heparin is observed during these interactions. Binding of factor Xa to antithrombin III which reacted with thrombin and heparin is also decreased or abolished.

These results indicate that in the presence of heparin thrombin not only exposes rapidly a binding site on the inhibitor, but also causes a further change leading to the deletion of antithrombin III binding properties. This may explain adverse, thrombotic effect of heparin sporadically seen in vivo, and suggests that heparin should be applied with caution in patients with antithrombin III deficiency.

Katherine Whigham, P. W. Howie, C. D. Forbes and C. R. M. Prentice (University Departments of Gynaecology and Medicine, Royal Infirmary, Glasgow, U.K.): **The Relationship between Antithrombin III, Progressive Antithrombin Activity and Anti Xa Activity in Plasma.** **(151)**

In 30 normal subjects, progressive antithrombin activity, as measured by the rate of thrombin neutralisation in ancrod-defibrinated plasma, was compared with antithrombin III, as measured by radial immunodiffusion. No significant correlation was found between the two methods of antithrombin measurement ($r = -0.101$). Similarly, no correlation was found between progressive antithrombin activity and immunological measurements of α_2 macroglobulin and α_1 antitrypsin. These results were not changed by using thrombin purified by Amberlite 1RC50 chromatography in place of commercial thrombin in the clotting test. There was, however, a strong positive correlation between the measurements of progressive antithrombin activity using the commercial and purified forms of thrombin ($r = 0.78$, $p < 0.001$). In contrast, there was a positive correlation between antithrombin III and anti-factor Xa activity in plasma ($r = 0.48$, $p < 0.01$). There was no correlation between plasma anti Xa activity and α_2 macroglobulin or α_1 antitrypsin.

These results suggest that plasma antithrombin activity is a measure of the activities of several plasma proteins and that antithrombin III may not be the major determinant of antithrombin activity. There is little evidence that immunological assays of antithrombin III reflect total thrombin inhibitory capacity as measured by the biological assay. Caution must be exercised in extrapolating from immunological measurements of antithrombin III to antithrombin activity in-vivo.