

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) Activity 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 is 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 exceed 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 exceed 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 ions 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.

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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 synthesized 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