ANALYSIS OF ANTICOAGULATION ACTIVITIES OF HEPARINS - IS USP HEPARIN ASSAY RELIABLE? Linus L. Shen and Grant H. Barlow, Abbott Laboratories, North Chicago, Illinois, U.S.A.

The *in vitro* "over-all" activity of heparins was analyzed by the technique of rigidity measurements with plasmas upon recalcification. This technique allows the measurement of the entire clotting process of a heparinized plasma, and is thus more sensitive and reliable than clotting time determination. Contrary to its action to the clotting of whole blood, heparin reduces the final rigidity of a plasma clot only when the degree of heparinization exceeds certain limit. A log-log plot of clotting half-time vs. amount of heparin may be used to compare the anticoagulation activity of various heparins in human plasma and in sheep plasma. Results show that heparin with molecular weight around 20,000 possesses highest specific activity, the activity drops sharply when molecular weight increases or decreases. The anticoagulation activity of heparin in human plasma, expressed by the increase in clotting half-time is up to 100 times more effective than that in sheep plasma but responds less sensitively to the change in heparin concentration. Using the same heparin standard, the specific activity of certain heparin fractions assayed in human plasma differs from that assayed in sheep plasma. The discrepancy increases with the decrease in heparin molecular weight. The discrepancy was also observed with some heparins of different tissues and sources. The USP heparin assay, which uses sheep plasma as the assay medium, therefore does not necessarily reflect the true activity in human blood clotting system.

THE HEPARIN INHIBITING PROPERTY OF BRAIN THROMBOPLASTIN. E.D. Gomperts and M. Zucker, Department of Haematology, School of Pathology of the University of the Witwatersrand and The South African Institute for Medical Research, Johannesburg, South Africa.

Antithrombin III is one of the serine proteinase inhibitors of the plasma which has been shown to specifically inhibit thrombin as well as Factor X. Heparin acts via antithrombin III, the heparin cofactor, hence it is difficult to explain the relative insensitivity of the prothrombin time to the presence of heparin in plasma as both thrombin, and Factor X are associated functionally with the prothrombin time. This insensitivity becomes more obvious on appreciating the extreme sensitivity to heparin of the activated partial thromboplastin time as well as the thrombin time. This communication reports the demonstration of heparin inhibiting action of brain thromboplastin. The response of the prothrombin time to heparin under various conditions, and the effect of brain thromboplastin obtained from various sources and by different preparative techniques on the action of heparin *in vitro* have been studied. The heparin inhibiting activity was shown to parallel the tissue factor activity. It is heat labile, non-dialysable, destroyed by detergent activity and lies in a high molecular weight fraction of the brain thromboplastin preparation ($> 300,000$). In addition to explaining certain *in vitro* phenomena, these observations may explain the previously observed heparin resistance in the generalised Schwartzman phenomenon.

THE ACTIVITY OF HEPARIN PREPARATIONS STUDIED BY AMIDOLYTIC AND CLOTTING METHODS. A.N. Teien, U. Abildgaard, M. H88k and U. Lindahl, Aker Hospital, Oslo, Norway and Royal Veterinary College, Uppsala, Sweden.

Two heparin standards, heparin isolated from human mastocytoma tissue, four commercial heparins and two heparin preparations separated by affinity chromatography ("High affinity heparin"=HAH and "Low affinity heparin"=LAH) were assayed by the activated partial thromboplastin time method (APTT), the calcium thrombin time method (CaTT) and two amidolytic methods (measuring the accelerating effect of heparin on the inactivation of thrombin or factor Xa by antithrombin III), with and without plasma in the test system. The specific activities of the various heparins were expressed relative to that of the 3rd. Int. Standard (=100). Found specific activities ranged 3 - 198 (LAH and HAH, respectively). In all assay systems HAH had the highest specific activity, followed by one of the commercial preparations and the 3rd Int. Standard. LAH and human heparin had very low specific activities, except in the APTT test system, an assay method which in addition mirrors other anticoagulant effects of heparin than the acceleration of antithrombin III. Apart from the higher effect of LAH and human heparin on the APTT, the difference in specific activities found for each individual heparin preparation with these various assay methods was slight.

In view of the reproducibility and simplicity of the amidolytic methods, it is suggested that they be adapted for heparin standardization.