MOLECULAR WEIGHT DISTRIBUTION STUDIES ON HEPARIN. Grant H. Barlow, Protein Chemistry Laboratory, Abbott Laboratories, North Chicago, Illinois, 60064, U.S.A.

The determination of molecular weight distribution using the sedimentation equilibrium analysis developed for polymers by T. Scholte (J. Polymer Sci, 6, 111, 1968) has been adapted for heparin analysis. Pork mucosal heparin separated into molecular weight subfractions by gel filtration on Ultrogel AcA44 (L.K.B.) was used to test the validity and resolving power of the method. Results indicate that the method is able to differentiate molecular weight distribution satisfactorily. Comparisons have been made of molecular weight distribution of samples from different species, organs and manufacturers. Average molecular weights for most samples center around 15,000 Daltons, but samples show considerable variation in their distribution data. Results suggest that variations between manufacturers is more pronounced than the species and organ difference indicating the importance of the purification procedure.

ANTICOAGULANT ACTIVITIES OF LUNG AND MUCOSAL HEPARIN. T.W. Barrowcliffe, E.A. Johnson, C.A. Eggleston and D.P. Thomas. National Institute for Biological Standards and Control, London, U.K.

Interaction with antithrombin III is thought to be the main mechanism whereby heparin exerts an anticoagulant effect, but measurements of this specific heparin activity by an anti-Xa assay do not always agree with measurements made by 'multiple role' assays, such as APTT or pharmacopoeial assays. Two batches of lung heparin had APTT activities *in vitro* about 1.4 times those found by anti-Xa, whereas in several batches of porcine mucosal heparin this ratio was about 0.8. All assays by both methods were carried out against the 3rd International Standard for heparin. After gel filtration, lung heparin maintained a high ratio of APTT to anti-Xa activity in all except the low molecular weight fractions, where the two activities were both about 60 i.u./mg. In contrast, low molecular weight mucosal fractions had negligible APTT activity, but high (120 i.u./mg) activity by anti-Xa assay. A nominal 1000 units of lung heparin injected I.V. into volunteers gave peak anti-Xa levels of about 0.2 i.u./ml; a comparable injection of mucosal heparin gave peak levels of about 0.3 i.u./ml. The resulting ratio agreed with the anti-Xa activities of these two batches *in vitro*. However, *in vivo*, APTT levels with both heparins were less than half the anti-Xa levels, and 50 mins. after injection there was virtually no effect on the APTT, while heparin levels by anti-Xa remained about 0.1 i.u./ml. Although their APTT activities were comparable, lung heparin had much less anti-Xa potentiating effect than mucosal heparin, both *in vitro* and *in vivo*; this has important implications for the assay and clinical use of heparin.

EXPERIENCE WITH AN AMIDOLYTIC HEPARIN ASSAY METHOD. A.N. Teien, U. Abildgaard, K. Gjesdal and A.H. Holm. Medical Department A, Aker Hospital, Oslo, Norway.

The amidolytic method using Xa, purified antithrombin III (At-III) and the chromogenic substrate Bz-Ile-Glu-Gly-Arg-pNA (S-2222 from KABI, Stockholm, Sweden) measures heparin down to a concentration of 0.010 U/ml plasma. The accuracy of the method was evaluated by adding heparin to plasma samples from 10 normals and 10 patients, to concentrations of 0.05 and 0.5 U/ml. The results indicated that the content was assayed more accurately than by existing clotting assays. The precision was 5 and 2 per cent of the mean value at the two heparin concentrations, respectively. The concentrations of platelet factor 4 and At-III in test plasma had some influence on the result at low heparin concentrations. The "error" due to At-III, however, may be corrected by a simple formula. Heparinized plasma (containing less than about 15 000 platelets/ μ l) could be kept at 0 - 4 °C for 24 hours before assay, kept frozen and thawed repeatedly without significant loss of heparin activity.

In plasma from patients on heparin prophylaxis with 5 000 U twice daily, heparin was detected in 98 per cent of the samples (range 0.010 - 0.241 U/ml).

The present assay method reflects heparin concentration more directly than the activated partial thromboplastin time test and the thrombin clotting time test. In order to investigate to what extent these three tests mirror the clinical effects of heparin (therapeutic response and bleeding complications) a collaborative study involving 14 hospitals has been started.