

FREE COMMUNICATIONS V

Thrombosis: Heparin Purification and Properties.

PREPARATION AND APPLICATIONS OF 125-I LABELLED HEPARIN. D.S. Pepper and Joan Dawes S.E. Scotland Regional Transfusion Service and MRC Endocrinology Unit, Edinburgh, Scotland.

Commercial heparin was derivatized with Bolton and Hunter reagent (SHPP, succinimidyl-hydroxyphenylpropionate) and the reaction products chromatographed on protamine agarose. Biologically active heparin eluted at 1.2 - 1.4 M NaCl and was iodinated by the chloramine T method, giving a product of specific activity 20 uCi/ug. The labelled heparin co-chromatographed with cold, biologically active heparin on protamine agarose. Using 0.4ng labelled tracer and 10^2 protamine agarose beads diluted in bland gel, it was possible to measure heparin concentrations of 0.001 u/ml by inhibition of binding. Heparin tracer alone eluted from 6% agarose molecular sieve columns earlier than heparin + plasma, indicating that the hydrodynamic radius of heparin is reduced when bound to AT III under physiological conditions. When injected intravenously, the label showed complex kinetics and did not have a single half life, clearance was initially rapid, but slowed later. Between 80-160 minutes following injection, the levels of circulating radioactivity rose slightly before falling again. This effect was reproducible in different normal persons and was independent of added heparin over a 100 fold range of dose.

STUDIES ON FRACTIONATED HEPARIN. D.A. Lane, I. Macgregor, R. Michalski and V.V. Kakkar. Thrombosis Research Unit, King's College Hospital Medical School, London.

Commercial porcine heparin has been divided into five different molecular weight components following gel filtration upon a polyacrylamide-agarose gel matrix. Rechromatography has shown that the two extreme fractions, which contain very low and very high molecular weight material, were totally separable upon gel filtration, while the intermediate fractions contained material in common with other fractions. Four of the five fractions contained almost equivalent specific activity when measured by an anti-Factor Xa clotting assay (1) and yet had very different specific activities if assayed by either thrombin clotting time or kaolin-cephalin clotting time methods. The highest MW fraction had low specific activity in all of the clotting assays, suggesting that it contained the greatest percentage of a non-reactive material demonstrated by Rosenberg et al, (2) while the lowest MW fraction had low activity only in thrombin clotting time and KCCT assays. The lowest MW fraction produced a smaller acceleration of purified fibrin monomer polymerisation rate and also smaller inhibition of thrombin induced platelet aggregation in plasma. The demonstration of different results for specific activities of the heparin fractions, depending upon the assay method used is in agreement with a recent study (3) and has important implications in regard to the pharmacopeal assays for heparin. It also suggests that heparins with higher specific anti-Factor Xa (or antithrombotic) effect can be prepared from commercial heparins, although these may have a relatively low ability to neutralise thrombin in plasma.

1. Yin, E.S., Wessler, S. and Butler, J.B. J.Lab.Clin.Med., 81, 289, 1973.

2. Lam, L.H., Gilbert, J.E. and Rosenberg, R.D. Biochem.Biophys.Res.Comm., 69, 570, 1976.

3. Andersson, L. et al. Thrombosis Res. In Press, 1977.