

FREE COMMUNICATIONS

14.00 – 16.00

Heparin Analogues and Fractions

Queen Elizabeth Hall

0996 SPIN PROBE SPIN LABELED STUDIES OF HEPARINS AND DERIVATIVES

S. E. Lasker*, I. Pullman[†], R. Pechauskas[†], and C. Patel, Department of Medicine, New York Medical College and Department of Dentistry[†], New York University Medical School, New York, New York, U.S.A.

The polyelectrolyte heparins have a multiplicity of activities *in vivo* and *in vitro* including interactions in the coagulation cascade. The stereochemical factors or functional groups specifically associated with these activities have not been clarified. In order to further study the structure-function relationships we used a probe technique, labeling the carboxyl groups of heparins, derivatives and chondroitins with ATEMPO. Electron spin resonance and biological activity studies of nitroxide labeled heparins, and derivatives reveal a small correlation time (τ_c) indicating that the label has free rotation when complexed to the carboxyl group. The labeled compound retains 85% of its anticoagulant activity and the complex binds antithrombin III to the same extent as heparin. Nuclear magnetic resonance spectra of the derivatives indicate that the complexing agent, carbodiimide is differentially displaced by ATEMPO at some of the carboxyl groups. The technique permits study of the stereochemistry of the carboxyl groups present in the polysaccharide.

14.15 0997 ISOLATION AND CHARACTERISTICS OF HIGH-ACTIVITY HEPARIN

Isidore Danishefsky*, Steven Radoff, Michael Bender and German Villanueva, Department of Biochemistry, New York Medical College, Valhalla, New York, U.S.A.

Chromatography of heparin from beef lung on Biogel P-100 yielded a series of fractions with different anticoagulant activities. The assays for activity of the products were based on their effect in accelerating the inhibition of thrombin by antithrombin. Residual thrombin was measured by its effect on the clotting of fibrinogen and by its action on the chromogenic substrate H-D-Phe-Pip-Arg-p-nitroanilide (S-2238). Fractions with as much as 6-10 times the clotting activity of the original heparin, were obtained. The activities per mg of heparin were highest in fractions eluted in, or near, the void volume of the column. In addition to glucosamine and uronic acid, the most active fractions contained considerable amounts of protein. The amino acids included glycine, serine, threonine, alanine, aspartate and glutamate. Experiments with heparin from porcine intestinal mucosa gave similar results.

Affinity chromatography of the highly active fractions on antithrombin-Sepharose showed that all of these materials are bound to the gel and that relatively large proportions are eluted with 1 M and 3 M NaCl.

It is concluded that the common heterodisperse heparin preparation contain a series of components ranging from extremely high activities to no activity. On the basis of the elution volumes of the fractions, the activities are directly related to molecular weight.