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PHYSICO-CHEMICAL STUDIES ON OLIGOSACCHARIDES FROM PROCINE MUCOSAL HEPARIN. J. Choay, J. C. Lormeau, M. Petitou, G. Torri and P. Sinay. Choay Institute, Paris, France, Istituto Ronzoni, Milan, Italy and Laboratoire De Biochimie Structurale, Uer De Sciences Fundamentales Et Appliquees, Orleans, France.

Our recent studies have revealed that low molecular weight antithrombotic (in vivo) and anti Xa fractions and fragments can be obtained from porcine mucosal heparin (PMH) using three different processes: 1) Direct alcoholic fractionations, 2) depolymerization with nitrous acid and 3) by bacterial heparinase digestion. The crude products are further fractionated by antithrombin-III affinity chromatography and gel filtration. Physiocochemical studies were carried on each fragment employing nitrous acid degradation, colorimetric and NMR analysis. A potent antithrombotic octasaccharide (h-ULMF) is isolated from heparinase depolymerized fractions and in the NMR analysis gave some unexpected signals one of which was consistant to a 3-O-sulfated glucosamine unit (unsaturated sulfated uronic acid/N-Sulfo-Glucosamine/Iduronic acid/N-Acetyl-Glucosamine/Glucouronic acid/N-Sulfoglucosamine/Sulfated Idouronic acid/N-Sulfo-Glucosamine). ULMFs obtained by extraction and clemical degradation were also found to exhibit similar signals. Further degradation of h-ULMF-8 resulted in formation of smaller fragments with biological activity. Our results suggest that a smaller oligosaccharide fragment contained in the biologically active octasaccharide molecule is primarily responsible for the antithrombotic actions. Furthermore these studies provide additional data on the structure activity relationship for the antithrombotic actions of heparin, its fraction and fragments.

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EFFECT OF SOME EXPERIMENTAL CONDITIONS ON THE ACTIVITY OF A LOW MOLECULAR WEIGHT (LMW) HEPARIN FRACTION COMPARED TO A HIGHT MOLECULAR WEIGHT (HMW) FRACTION. M. Aiach, C. Nussas and J. Mardiguian. Département d'Hémostase, hôpital Broussais, Paris et département de recherche, groupe Pharmuka, Gennevilliers (France).

In this work, we aimed to demonstrate that different methods can give different results when the same pair of heparin samples are compared, even when specific antiprotease assays are performed. For this purpose, the effect of heparin on factor Xa (Xa) or thrombin (IIa) inhibition by antithrombin III (AT III) was examined in the presence of varying amounts of AT III, during different incubation times.

The molecular weight of the two heparins were 5,200 (LMW) and 42,000 (HMW). Solutions containing 2 µg per ml of heparin and a 1.4 to 15 µM freshly purified human AT III were incubated with either bovine Xa or human IIa. After a 20, 30, 60 or 90 secondes incubation at 30° C, the remaining protease activity was measured by the initial velocity of a chromogenic substrate. The method was entirely automated using a reaction rate analyser and an adapted program.

The antiprotease activity of the LMW heparin (related to the HMW heparin activity) varied from 0.23 to 0.89 in the anti Xa system, from 0.30 to 0.77 in the anti IIa system. The ratio of LMW to HMW activity was a parabolic function of either AT III concentration or incubation time. No meaning differences were observed between anti Xa and anti IIa activity when the inhibiting capacity was assayed in the same experimental conditions. These results suggest that the relative activities of HMW and LMW fractions depend upon the assay procedure. AT III concentration as well as incubation time are of particular importance.

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HEPARIN CONCENTRATIONS CORRELATED TO THE ACTIVATED WHOLE BLOOD CLOTTING TIME (ACT) DURING EXTRACORPOREAL CIRCULATION. S. Stenbjerg, E. Berg and O.K. Albrechtsen. Coagulation Laboratory, Blood Bank and Tissue Typing Laboratory, Department of Cardiovascular Surgery, University Hospital, Aarhus, Denmark.

Heparin levels and ACT were followed during open heart surgery in 10 patients. Heparin was assayed by an amidolytic method using substrate S-2222. ACT was determined with an automated method using celite and glass beads as activators of coagulation. Neither the hemodilution nor the depletion of platelets observed during extracorporeal circulation seemed to influence the ACT. An excellent correlation between the ACT and the actual heparin level was found in each patient with coefficients of correlation ranging from 0.73 - 0.97. A slightly better correlation was noticed for values of ACT below 600 seconds. It was concluded that the ACT is a valuable and reliable tool in control of heparinisation during open heart surgery.

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HEPARIN THERAPY IN *DISPHOLIDUS TYPUS* ENVENOMATION: AN EXPERIMENTAL STUDY. B.A. Bradlow, P.M. Atkinson, M. Rebello and M.C. Gaillard. Department of Haematology, School of Pathology of the South African Institute for Medical Research and The University of the Witwatersrand, Johannesburg, Republic of South Africa.

The coagulant action of *Dispholidus typus* venom was relatively resistant to inhibition by heparin *in vitro*. Heparin concentrations that inhibited coagulation due to either intrinsic pathway or Russell's viper venom activation had little effect on coagulation due to D. Typus venom. At very high heparin to venom ratios, similar to ratios attainable *in vivo*, this resistance could be overcome. The resistance could not be attributed to an abnormal thrombin produced by the venom since the thrombin produced from purified prothrombin by venom action reacted similarly to the thrombin produced by Factor Xa activation with purified antithrombin III. Thrombin produced from whole plasma by venom action also reacted similarly to physiological thrombin with antithrombin III in a crossed immunoelectrophoresis system. Incubation of venom with heparin and with antithrombin III did not alter the activities of these inhibitors. The heparin resistance may therefore be due to the fact that the venom is a direct activator of prothrombin. *In vivo* studies in rabbits indicated that heparin administered simultaneously with venom delayed the onset and reduced the severity of disseminated intravascular coagulation. Heparin administered later was much less effective. Early heparin therapy may be of value in human victims when specific antivenom is not available.