Friday, July 17, 1981

Poster Presentations

Antithrombin III – I

11:00–12:30 h

Kent Room Boards 137–148

0893

PREPARATION OF ANTITHROMBIN, HIGH ACTIVITY HEPARIN AND A HEPARIN-ANTITHROMBIN COMPLEX BY A RAPID TWO-STEP AFFINITY METHOD. R. Jordan, T. Zuffi, M. Fournel, and D. Schroeder. Biochemical Research Dept., Cutter Laboratories, Inc., Berkeley, California.

The tight binding affinity of antithrombin for heparin makes possible a relatively selective purification scheme based on salt elution from heparin-Sepharose. We have found, however, that purity can often be greatly increased if the elution is carried out with soluble heparin instead. This heparin can be removed from the antithrombin, either in whole or part, by a second affinity step on Concanavalin A Sepharose. The antithrombin, which binds to the matrix through its glycosidic moieties, retains its ability to bind heparin at physiological ionic strengths. Thus, the complex of antithrombin and heparin is readily isolated free of unbound heparin species. The complex can be eluted intact with low ionic strength buffers containing sugars which compete for binding to the lectin. Alternatively, the high activity heparin (400–500 units/mg) can be obtained separately by a 1 M NaCl wash which is then followed by a carbohydrate wash to obtain the purified antithrombin.

We have made certain preliminary biochemical and anticoagulant characterizations of these materials. Not unexpectedly, both the high activity heparin and its complex with antithrombin show significantly greater *in vitro* potency in comparison to unfractionated heparin. *In vivo* anticoagulant efficacy, as evaluated in a rabbit infusion model, confirmed the *in vitro* findings and further suggests some potential therapeutic benefit may be derived from infusion of a preformed heparin-antithrombin complex.

0894

IDENTIFICATION OF THE AT III SYNTHESIZING HEPATOCYTES BY IMMUNOFLUORESCENT TECHNIQUE. M. Watada, M. Nakagawa, T. Kitani, Y. Okajima, Y. Maeda, S. Urano and H. Ijichi. Second Department of Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Antithrombin III (AT III), heparin cofactor has been known to be one of the important protease inhibitors on the regulatory mechanism of coagulation and fibrinolysis, but the site of synthesis of plasma AT III has not yet been conclusively established, although some observations suggested that the major site of AT III production may be the liver and AT III levels in plasma is known to be decreased on the hepatic disorders. This paper reports the deposition of the AT III specific fluorescence in the hepatocytes of rat liver by immunofluorescent examination. Plasma AT III was purified by heparin sepharose affinity chromatography and gel filtrations. Rabbit was immunized with this purified AT III and Freund's complete adjuvant and rabbit anti rat AT III serum was obtained. This antiserum was comfirmed to be monospecific to rat AT III by means of immunoelectrophoresis. Freshly isolated rat liver was prepared as specimen and fixed in acid alcohol and processed to dehydration and embedding into paraffin in conventional way. Indirect immunofluorescent technique with FITC labeled anti rabbit gamma-globulin were utilized for immunofluorescent staining and histological observation was performed under Nikon FL fluorescent microscope. The localization of fluorescence was observed in the hepatocytes which scattered in the acinus structures. The fluorescence in the cytoplasma of these hepatocytes was homogeneously stained. As far as the fluorescence is concerned, the synthesis of AT III may be performed not synchronizing among hepatocytes. This immunofluorescent study supports the reported data of hepatic perfusion.