

ISOLATION OF THE ANTITHROMBIN-THROMBIN COMPLEX AND PLATELET FACTOR 4 BY SOLID PHASE HEPARIN. H. Blevl and L. R6ka, Justus Liebig-University, Dept. of Clinical Chemistry, Giessen, West-Germany

Solid-phase-heparin as heparin-agarose [Hep-Ag] was used for the isolation of the complex formed by antithrombin and thrombin. This complex has a lower affinity to heparin than antithrombin III. The complex has no antithrombin activity. The increase of the complex during blood coagulation can be seen by the two-dimensional immunoelectrophoresis in agarose, which contains heparin (Sas et al., Thromb.Res. 6, 87-91, 1975).

Platelet factor 4 (PF 4) was isolated by a three step procedure: 1. precipitation by divalent cations according to Burstein, 2. adsorption to Hep-Ag and desorption with high salt concentrations, 3. desalting over Sephadex G 25 fine. PF 4 was prepared from isolated platelets, platelet rich plasma and serum.

ANTITHROMBIN FUNCTIONAL ACTIVITY IN NORMAL SUBJECTS AND PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA AND NEPHROTIC SYNDROME. N. Zucker, E.D. Gomperts, D. Russel, D. Salant, M. Feesey, H. Seftel and D. Mendelsohn. Department of Haematology and Lipid Research Unit of the School of Pathology, University of the Witwatersrand and S.A.I.M.R., Johannesburg, S.A.

Nineteen patients with familial hypercholesterolemia, 5 patients with hyperlipidaemia secondary to nephrotic syndrome and 14 normal individuals were given a meal containing 1g saturated fat/Kg body weight. The plasma total functional antithrombin (AT) activity was measured in the normal before the meal and 2, 4 and 6 hours after the meal by both a clotting assay system and the chromogenic substrate S2160. Only the clotting assay system could be employed in the hyperlipidaemia situation. The functional plasma AT-III activity was compared with AT protein levels measured by quantitative radial immunodiffusion. Functional AT activity was depressed maximally at 2 hours after the meal in 13 of the normal and all of the nephrotic individuals studied. The immunologically measured AT-III levels remained constant throughout the experiment. Only 9 of the 19 familial hypercholesterolemic patients showed a similar phenomenon. The functional depression in AT activity failed to correlate with the rise in serum triglyceride level. Basal functional AT activity was similar in the normal and hypercholesterolemia patients but considerably increased in the nephrotic patients studied. When similar studies with an unsaturated fatty meal were carried out in normal volunteers no significant alteration in functional AT activity was observed. In addition, 11 volunteer normal individuals were fasted for 24 hours. Considerable increases in functional AT activity were present in 9 of the 11 subjects. These studies indicate that functional AT activity may be modified by various physiological and pathological factors.

LARGE SCALE METHOD FOR THE ISOLATION OF ANTITHROMBIN III. M. Wickerhauser and C. Williams, Blood Research Laboratory, American National Red Cross, Bethesda, Maryland, USA.

Antithrombin III (AT III) concentrates may be of value in the treatment of various hypercoagulable states associated with congenital or acquired AT III deficiency. We have developed a large scale method for isolation of AT III from plasma or from Cohn fraction IV-1, an unused byproduct of routine plasma fractionation. The method consists of the following steps: (a) precipitation of the starting plasma or Cohn fraction IV-1 extract with 20% polyethylene glycol (PEG) 4000 to remove a number of impurities including hepatitis B antigen if present; (b) batchwise adsorption to and elution from heparin-Sepharose using one volume of gel for each 50 liters of the 20% PEG supernatant; and (c) concentration of the eluted AT III by bulk lyophilization, followed by desalting on Sephadex G-50, sterile filtration and final lyophilization. In two large scale experiments, 7.1 and 8.7 grams of AT III, determined as antigen by radial immunodiffusion, corresponding to 25,000 and 31,000 plasma equivalents, were recovered from 100 liter plasma and 15 kg Cohn fraction IV-1 batches respectively. Both preparations were over 95% pure by disc gel electrophoresis and had an activity to antigen ratio close to that of AT III in plasma. Both preparations were nonpyrogenic and met all other FDA requirements for biologic products.