Physiological Reactions of Antithrombin III

Hungerford Room

Time

14.00 **0400** ISOLATION OF ANTITHROMBIN III WITHOUT INTERFERING WITH ETHANOL FRACTIONATION SYSTEM

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We described previously the isolation of antithrombin III (AT III) from the 20% polyethylene glycol (PEG 4000) supernatant of plasma or of Cohn Fraction IV-1 (Vox Sang., in press). The first of these two methods gives good recoveries of AT III but cannot be integrated with the conventional ethanol fractionation system due to the presence of PEG in the remaining plasma fraction, while Cohn Fraction IV-1, a byproduct of routine fractionation, is a poor source of AT III in terms of yield. Our modified method involves batchwise adsorption of AT III from plasma (cryosupernatant) with heparin-Sepharose, using one volume of gel for each 50 volumes of plasma. The unadsorbed plasma can be used for ethanol fractionation. The AT III eluate is further purified by precipitation of some impurities including HB_sAg, if present, with 20% PEG. Final purification of AT III and removal of PEG is achieved by a second adsorption-elution step on heparin-Sepharose. This method is economical and suitable for large scale application. Recovery of a highly purified AT III was 25%.

14.15 **0401** PLASMA INHIBITION OF THE HEPARIN ACCELERATED NEUTRALISATION OF Xa BY ANTITHROMBIN III

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The ability of heparin fractions of different MW (mean MW 8500-30,000) to potentiate the action of antithrombin III against the coagulation factors thrombin and Xa has been examined in purified reaction mixtures and in plasma. Residual thrombin and Xa have been determined by their peptidase activities against the synthetic peptide substrates H-D-Phe Pip-Arg-pNA and Bz-Ile-Glu-Gly-Arg-pNA. High MW heparin fractions were found to have higher anticoagulant activities than low MW heparin when studied with both thrombin and Xa incubation mixtures in purified mixtures. The inhibition of thrombin by heparin fractions and antithrombin III was unaffected by other plasma components. However, normal human plasma contained a component that inhibited the heparin and antithrombin III inhibition of Xa, particularly when the high MW heparin fraction was used. Experiments using a purified preparation of platelet factor 4 suggested that this platelet derived heparin neutralising protein was not responsible for the inhibition and that a hitherto undescribed inhibitor of heparin action is present in plasma. Preliminary studies have indicated that the inhibitor is concentrated in a fraction rich in lipoprotein and obtained by ultracentrifugation of plasma in high density salt.