

**Monday, July 13, 1981**

## **Poster Presentations**

### **Heparin – I**

#### **Antithrombin III**

**11:00–12:30 h**

**Kent Room Boards 137–148**

#### **0114**

THE EFFECTS OF HEPARIN ON THE ACTIVATION OF FACTOR X AND PROTHROMBIN IN ANTITHROMBIN III-DEPLETED PLASMA. F. Ofosu, A. Cerskus, J. Hirsh and M.A. Blajchman. The Canadian Red Cross Blood Transfusion Service and the Department of Pathology, McMaster University, Hamilton, Ontario, Canada.

The predominant mode of the anticoagulant action of heparin is considered to be the enhancement of the rate of inactivation, by antithrombin-III, of several activated clotting factors. Recent evidence, however, suggests that heparin can reversibly inhibit the activation of prothrombin and factor X even in the absence of antithrombin-III. This antithrombin-III-independent action of heparin has been demonstrated only in purified clotting factor systems. In order to determine the significance of the antithrombin-III-independent effects of heparin in plasma, the effects of heparin on the activation of factor X and prothrombin were studied in antithrombin-III-depleted plasma produced by affinity chromatography of normal plasma on heparin-Sepharose. Heparin partially inhibited the activation of factor X and prothrombin on the addition of either factor IXa or factor Xa in antithrombin-III-depleted plasma. This inhibition was demonstrable only when high concentrations (1 or 10 units/ml) of heparin were used. In contrast, when as little as 1% antithrombin-III was added to antithrombin-III-depleted plasma containing 1.0 u of heparin per ml of plasma, no factor X or prothrombin activation could be demonstrated. Thus, it appears that in comparison with the magnitude of the antithrombin-III-dependent effect, the contribution of the antithrombin-III-independent anticoagulant effect of heparin on the activation of factor X and prothrombin in normal plasma is limited.

#### **0115**

ANTITHROMBIN ASSAY – A STUDY OF REACTION CONDITIONS, REAGENTS AND KINETICS. P. Friberger, M. Knös and M. Andersson. Kabi AB, Peptide Research, Mölndal, Sweden.

Antithrombin (AT) in plasma diluted as much as 1:600 in the final incubation mixture reacts rapidly and essentially quantitatively with thrombin in the presence of 3 IU/ml of heparin using tris buffer pH 8.3 and I 0.15. Normal plasma levels of AT consume approximately 60% of human thrombin when 7 nmol/l is added. The reaction is similar to a 2nd order reaction,  $k_2 = 8 \cdot 10^6$  l/mol·sec and is almost complete after 3 min incubation. The excess amount of thrombin is then determined with the chromogenic substrate S-2238.

Data collected prove: 1. The total amount of the AT co-factor activity is titrated. 2. Other plasma inhibitors do not interfere. 3. The quality and/or source of thrombin and heparin is of definite importance. 4. The procedure for preparing the standard curve and the selection of standard is critical. 5. Reagents with good stability can be selected.

The assay is performed in two steps but easy handling is obtained by the use of the same volume of each reagent and the selection of suitable incubation times – all in order to allow reproducible results also in a routine laboratory. This is important since there are small differences between normal and pathologic values.