PURIFICATION AND METABOLISM OF RAT ANTITHROMBIN III. S.Okuda, M.Nakagawa, T.Okada, K.Sawada, and H.Ijichi. Kyoto Prefectural University of Medicine, Kyoto, Japan.

The previously reported heparin sepharose affinity column has been proved to be reduced in its recovery on the repeated uses because of loose binding of ligand to sepharose. We tried to obtain the better recovery of antithrombin III (AT-III) using four different affinity columns of CNBr activated sepharose 4B, AH-sepharose 4B, CH-sepharose 4B, and Epoxy activated sepharose 6B. ^3H -labeled heparin was used to check its binding capacity to sepharose gels. Binding of heparin to gel surface was found to be tight in AH-sepharose 4B, CH-sepharose 4B, and Epoxy activated sepharose 6B because of the difference of the binding sites of heparin free radicals but their recovery and purity of AT-III were poor.

According to this experimental results, CNBr activated sepharose 4B was used for the purification of rat tissue AT-III and the metabolism of AT-III was investigated from the stand point of ^{14}C -glycine incorporation into AT-III fractions. ^{14}C radioactivity in the tissue AT-III fractions were observed immediately after injection and reached to the maximum at 6 hours and appeared to be released into circulating pool in 2 hours after injection.

INCREASED PROCOAGULANT ACTIVITY OF PHOSPHOLIPID IN THROMBO-EMBOLIC DISEASES. M.C. Boffa, D. Gozin. Centre National de Transfusion Sanguine, Paris, France.

Procoagulant activity of phospholipid has been measured in recalcified plasma as previously described (1), by means of a viperidae anticoagulant phospholipase A₂, specific inhibitor of phospholipid in clotting (2).

With reference to a control normal group and to a group of atherosclerotic patients, phospholipid activity was found significantly increased in thrombo-embolic diseases, with maximum frequency after recent embolism. In the oral contraceptives group, this assay appeared as useful for selecting women predisposed to thrombosis: in fact, about 5% of the treated women showed a high phospholipid activity contrary to the control group. One month after interruption of the oestrogen treatment, the phospholipid activity became normal. The few cases examined during thrombosis presented a very high activity.

In patients with high plasma phospholipid content (hyperlipoproteinemia) phospholipid activity was practically normal. This shows that procoagulant activity of phospholipid is related only to a very small fraction of phospholipid, independant of the lipoprotein phospholipid and very likely from platelet origin.

(1) *Thromb. Res.* 1974, 4, Suppl. 1, 89-90.

(2) *Biochim. Biophys. Acta* 1976, 429, 828-852.

THE INFLUENCE OF PIVKA II ON THROMBIN GENERATION IN LIVER DISEASE. R. G. Malia, and F. E. Prenton. University Department of Haematology, Royal Infirmary, Sheffield, England

Thrombin generation and factor Xa activation have been studied in plasma samples from normal controls and from patients with various types of hepatic dysfunction. The patients were subdivided into groups according to the ratio procoagulant II/ PIVKA II. PIVKA II was measured by immunoelectrophoresis using a rabbit antibody raised against prothrombin. For those patients with a ratio greater than 0.70 the thrombin generation and Xa activation correlates well with the procoagulant factor II. Conversely patients with a ratio less than 0.5 exhibit inhibition within these same test systems. The administration of vitamin K abolishes this effect.

It is concluded that PIVKA II plays an important role in the coagulation mechanism of patients with hepatic dysfunction associated with vitamin K deficiency.