ANTITHROMBIN III: CRITICAL REVIEW OF ASSAY METHODS. SIGNIFICANCE OF VARIATIONS IN HEALTH AND DISEASE. O.R.Ødegård and U.Abildgaard, Aker Hospital, Oslo, Norway.

Thrombin and factor Xa which are added to plasma are inactivated by At-III. The reactions are accelerated by heparin, permitting assay systems which rapidly measure At-III content of diluted plasma. Without heparin, the (slow) inactivation [illegible] measured. The review of existing activity assays (fibrinogen or chromogenic substrates), and semiquantitative assays, will focus upon their usefulness and limitations, and to the correlation to results of immunoassay.

In health, a narrow range of At-III concentration (and activity) is found. The level is low in infancy. Fertile women have on the average lower levels than men. In old age the level tends to drop. In the normal material that will be reported, one man was the propositus of a "new" family with At-III deficiency and thrombophilia. In a clinical material (n=2000), studied with amidolytic assay, subnormal At-III levels were found in hereditary deficiency, liver cirrhosis, disseminated intravascular coagulation and in some cases with acute thrombosis. Plasma samples containing soluble fibrin (positive ethanol gelation test) were classified as to the degree of hypercoagulation according to the values of other coagulation tests (grade 1-4, grade 4=DIC). The mean At-III plasma level decreased with increasing degree of hypercoagulation. Due to its high accuracy and simplicity, we have chosen the amidolytic activity assay with heparin and thrombin (Thromb. Res. 6, 287, 1975) for routine work.

ANTITHROMBIN III AND ITS INTERACTIONS WITH HEPARIN. R.D. Rosenberg, I.R. Jordan, G. Armand, L.H. Lam and D.L. Beeler, Sidney Farber Cancer Institute, Boston, Massachusetts, U.S.A.

Heparin functions as an anticoagulant by binding to antithrombin and accelerating the rate at which this protein inhibitor inactivates the serine proteases of the hemostatic system. Despite considerable effort, the precise relationship between the structure of this mucopolysaccharide and its biologic properties has remained elusive.

We have provided the first evidence that only a small portion of a given heparin preparation binds tightly to antithrombin and is responsible for the bulk of anticoagulant activity. The major fraction of these products has very little anticoagulant effect.

In our presentation, we shall discuss optimal techniques for fractionating heparin into "highly active" and "relatively inactive" molecular forms. Furthermore, we shall consider the distinctive interactions of these two types of mucopolysaccharides with antithrombin, thrombin and other hemostatis system components. Lastly, we shall provide evidence that several structural differences exist between these two forms of heparin.

IN VITRO CHARACTERIZATION OF RABBIT THROMBIN, ANTITHROMBIN III, AND THROMBIN-ANTITHROMBIN III COMPLEX, AND DETERMINATION OF THEIR SURVIVAL TIMES IN VIVO. Christine N. Vogel, Henry S. Kingdon, and Roger L. Lundblad. University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, U.S.A.

Our intention is to study the interaction of rabbit thrombin with antithrombin III (AT-III) in vitro and in vivo. After activation of crude prothrombin with tissue thromboplastin and $CaCl_2$, thrombin was purified and showed two species of thrombin with molecular weights of 36,000 and 39,000 daltons as determined by sodium dodecyl sulfate discontinuous gel electrophoresis. Rabbit AT-III was purified using a heparin agarose column and had a molecular weight of 55,000 daltons. The inhibition of thrombin by AT-III was followed by fibrinogen clotting assays and an AT-III-thrombin complex was observed on gel electrophoresis. For the in vivo studies both thrombin and AT-III were radiolabelled with $Na^{125}I$ using the solid state lactoperoxidase method and retained 99% of the pre-iodinated specific activity. Radiolabelled thrombin and a radiolabelled AT-III-thrombin complex were injected into different rabbits. The rate of removal of both was very similar with a half-life of approximately 9 hours. When radiolabelled AT-III was injected, the half-life was approximately 60 hours. Since the disappearance rate of thrombin more closely approximates that of the preformed AT-III-thrombin complex and is clearly shorter than the turnover rate of AT-III, the possibility is raised that thrombin combines in vivo with a native inhibitor such as AT-III and may in fact be removed from the circulation as a complex rather than as a native molecule.