KINETICS OF THROMBIN INHIBITION BY ALPHA2 MACROGLOBULIN: CHANGES INDUCED BY PRIOR PROTEOLYSIS OF THE INHIBITOR BY TRYPSIN. J. Tapon-Bretaudiere and F. Josso, Department of Hematology, University-Hospital Necker Enfants Malades, Paris, France.

Plasma alpha2 macroglobulin ($\alpha 2M$) is able to inhibit serine esterases by a two-step reaction involving a first cleavage of the inhibitor molecule by the proteinase, followed by the formation of a molecular complex between the enzyme and the proteolyzed inhibitor. The rate of the inhibition reaction varies greatly from one proteinase to one another. Thus, $\alpha 2M$ appears to be a "progressive" inhibitor of thrombin.

Kinetics of thrombin inhibition by $\alpha 2M$ was evaluated after prior treatment of the inhibitor by trypsin, either immobilized to avoid the secondary fixation of the enzyme to the proteolyzed inhibitor, or soluble, the enzyme excess being neutralized by soybean trypsin inhibitor (STI). In the second case, trypsin bound to $\alpha 2M$ was assayed using a low molecular weight synthetic substrate (BAPNA).

When $\alpha 2M$ was pretreated by immobilized trypsin, the reaction rate of the inhibition was increased without modification of the over-all neutralizing activity of $\alpha 2M$.

When $\alpha 2M$ was pretreated by soluble trypsin, it was found that STI in excess was able to displace trypsin bound to $\alpha 2M$. In these conditions, the treated $\alpha 2M$ inhibited thrombin at a very rapid rate, the over-all neutralizing activity of $\alpha 2M$ depending on the ratio STI/Trypsin.

These findings demonstrate that (i) the binding of $\alpha 2M$ to proteinases is reversible (ii) the limiting factor of the inhibition rate of thrombin by $\alpha 2M$ is the first proteolytic step of the inhibition reaction.

FAMILIAL THROMBOSIS DUE TO ANTITHROMBIN III DEFICIENCY: AN EXTENSIVE FAMILY STUDY. Bruce Bennett, Michael Mackie, Alexander S. Douglas, Department of Medicine, University of Aberdeen, Scotland.

A family living in Lewis (a Hebridean Island off the north west coast of Scotland) affected by antithrombin III deficiency has been studied. Two members have died, one of massive pulmonary embolism and one of major mesenteric infarction secondary to mesenteric vein occlusion. A further individual has sustained major small bowel infarction secondary to mesenteric vein thrombosis but survived after two small bowel resections followed by anticoagulant therapy. Other members of the family have suffered from non-fatal thrombotic events particularly during pregnancy. 57 individuals representing several generations have been studied and will be presented. Levels of antithrombin III measured by functional assays correlated well with those of antithrombin III measured immunologically. 12 members of the family showed moderate to severe deficiency of antithrombin III, the occurrence of thrombotic symptoms correlating well with deficiency of this protein. Transmission of the disorder as an autosomal dominant disorder is confirmed in the patients studied and by historical evidence over many generations.

PRODUCTION BY HUMAN LUNG CELLS OF TRYPSIN AND PLASMIN INHIBITOR(S) DIFFERING FROM α_1 -ANTITRYPSIN, α_2 -MACROGLOBULIN, C_1 -ESTERASE INHIBITOR AND INTER- α -TRYPSIN INHIBITOR. Maria B. Bernik, Northwestern University Medical School, Chicago, Illinois, U.S.A.

Human lung cells in primary culture and serial subculture were used to study the production of inhibitory activity and to isolate and identify inhibitor(s) of trypsin and plasmin produced and released by the cells into the supernatant medium. Assays of inhibitory activity were performed on fibrin and casein substrate and results expressed in BAEE units of trypsin inhibited on the former substrate. Inhibitory activity against plasmin and trypsin accumulated progressively in serum free supernates of cultures to concentrations of 80-150 BAEE units/ml and was isolated from the supernates by concentration with Amicon PM 30 membranes, gel filtration on Sephadex G-100 or G-200 columns and polyacrilamide gel electrophoresis. On calibrated columns inhibitory activity eluted in the range of 75,000 mol wt substances. On immunodiffusion, performed using a wide range of concentrations of chromatographed inhibitor preparations with specific activity of about 1,300 BAEE units/mg protein there was no cross-reaction with anti-serum to α_1 -antitrypsin, α_2 -macroglobulin, C_1 -esterase inhibitor or inter- α -trypsin inhibitor. There was also no cross-reaction with antiserum to antithrombin III or antichymotrypsin. Immunoelectrophoresis showed no immunoreactive material with the antisera. These observations indicate the production in lung of inhibitor(s) differing from the major protease inhibitors and derivatives or subunits of these inhibitors described to date.