STUDIES ON A NOVEL CIRCULATING ANTICOAGULANT IN A FEMALE WITH BLEEDING DIATHESIS. Harry L. Messmore, J. Fareed, Roberta L. Chang, Gerald M. Gawlik and Gerald F. Kozuh, Loyola University Medical Center, Maywood, Illinois USA

A 42 year old Caucasian female has had a history of bleeding from surgical trauma since the age of 3. She has bled massively from surgical trauma and has had life threatening postpartum hemorrhage. Laboratory tests showed marked prolongation of thrombin time, prothrombin time, partial thromboplastin time, low α_1 antitrypsin and lipoprotein lipase activity. Fibrinogen level was normal. Mixing of patient's plasma and serum with citrated normal plasma suggested the presence of a potent antithrombin (AT) substance. Heating of both the plasma and serum at 56°C for 2 hours failed to destroy the AT activity. The AT activity of the patient's plasma was quantitated to be equivalent to 0.75-0.9 u/ml heparin. Platelets aggregated normally with ADP, collagen, epinephrine, but not with Ristocetin and thrombin (0.5-5.0 u/ml). Her plasma and serum blocked thrombin induced aggregation of normal platelets. Her plasma also inhibited the factor X_a induced hydrolysis of S-2222 (Bz-Ile-Glu-[γ -OR]-Gly-Arg-pNA) and the action of thrombin on S-2160 (Bz-Phe-val-Arg-pNA). The inhibitor was not adsorbed with barium sulfate or reduced in activity by protamine sulfate, toluidine blue or heparinase. It was chromatographed on Sephadex G-100 and G-50, and eluted in the void volume and a low molecular (10-20,000) fraction. Incubation of the patient's plasma with chymotrypsin and trypsin destroyed the anticoagulant activity. Although anti-AT-III serum totally neutralized the anticoagulant activity of heparinized plasma, it failed to neutralize the patient's plasma anticoagulant activity. Albumin partially neutralized the anticoagulant activity. These studies suggest that this AT substance is a potent inhibitor of serine proteases and behaves as activated AT-III.

INACTIVATION OF THROMBIN AND FACTOR X_a BY ANTITHROMBIN III AND PLASMA: STUDIES WITH BOVINE AND HUMAN ENZYMES. O.R. Ødegård and U. Abildgaard, Aker Hospital, Oslo, Norway.

The results of some earlier studies have indicated that At-III preferentially reacts with X_a rather than thrombin (1). We have estimated the inactivation rates using chromogenic substrates (2,3) to measure remaining enzyme activities after incubation with plasma or purified At-III. The enzyme half-time values were approximately inversely proportional to the At-III or plasma concentration. When added to purified human At-III or plasma, human X_a was more slowly inactivated than human thrombin. With an At-III activity of 70% of normal, the half-time of human thrombin was about 96 seconds, of human X_a about 150 seconds. Simultaneous incubation of At-III with human thrombin and X_a confirmed that thrombin was preferentially inactivated. Purified human At-III behaved similar to human plasma. The results indicate that with human reagents, At-III preferentially reacts with thrombin rather than with X_a . The enzymes of bovine origin were more rapidly inactivated than the human enzymes. Heparin accelerated the inactivation rates, but did not alter the order of inactivation rates between the enzymes.

1. Yin, E. T.: *Thrombos. Diath. haemorrh.* 33, 43, 1974.

2. Svendsen et al: *Thromb. Res.* 1, 267, 1972.

3. Aurell et al, 5th Int. Congr. on Thrombosis and Haemostasis, Paris 1975, p. 300.

EFFECT OF HEPARIN ON CIRCULATING ANTITHROMBIN III LEVEL. E. Marciniak and J.P. Gockerman, Department of Medicine, University of Kentucky, Lexington, Kentucky, USA.

Both the natural defense against thrombosis and the anticoagulant effect of heparin rely essentially on the presence of antithrombin III (AT III) in blood. The rationale for heparin therapy is to strengthen the existing inhibitory potential of AT III by increasing the velocity of binding. It has not been established whether there is a modifying effect of heparin on the circulating levels of AT III. Twenty-two patients with documented or suspected thromboembolism, including one with hereditary AT III deficiency, were studied before, during and after continuous intravenous infusion of 750-2000 heparin u/hr. AT III was evaluated in plasma 1) as a total factor X_a binding capacity in a method independent of the velocity of inhibition, 2) by immunodiffusion, 3) by rocket immunoelectrophoresis. In all patients, regardless of their initial AT III level, there was a marked progressive decrease in AT III during heparin infusion, with an abrupt return to original levels in 2-3 days after cessation of therapy. The average maximum decrease in the 5th-7th day of therapy was of 0.38 ± 0.09 u/ml (normal AT III = 1.0 u/ml) or 11.2 ± 2.5 mg/dl and was not affected by concurrent therapy with coumadin. Heparin given in repeated intravenous bolus injections also gradually lowered the quantity of circulating AT III but no changes were seen after a single intravenous injection in normal volunteers or subjects with congenital AT III deficiency. These results give evidence for a possible mechanism of a paradoxical, thrombosis inducing effect of heparin and indicate that low AT III levels in heparinized patients should not be mistaken for a cause or result of the underlying thromboembolic episode.