THE CARBOHYDRATE COMPONENTS IN THE ACTION OF ANTITHROMBIN III. I. Danishefsky, A. Zweben and B. Slomiany. New York Medical College, Valhalla, New York, U.S.A.

The carbohydrate components of human antithrombin III were identified and the contribution of the sialic acid component to the interaction of the inhibitor with thrombin and heparin was studied. Gas chromatographic analyses showed the following percent composition: galactose, 0.77; mannose, 1.49; N-acetylglucosamine, 1.68; sialic acid, 1.88, corresponding to a molar ratio of approximately 2:4:4:3, respectively. Colorimetric analysis by the thiobarbituric acid method, showed an average value of 3.3% neuraminic acid, indicating the presence of three residues of the latter per galactose unit. The neuraminic acid was removed by treatment with neuraminidase from Cl. perfringens, and the product was purified by affinity chromatography on heparin-aminohexylsepharose. Except for sialic acid, the modified antithrombin III had the same carbohydrate composition and circular dichroism spectrum (300-190 nm.) as the original inhibitor. The desialylated product retained its effect in the progressive inhibition of thrombin and in the rapid deactivation of the latter in the presence of heparin. (Thrombin activity was assayed by its effect on fibrinogen.) Gel electrophoresis experiments on the desialylated product also showed that it forms a complex with thrombin similar to that with native antithrombin III. It is concluded, therefore, that the sialic acid component is not involved in the binding of the inhibitor to thrombin or heparin.

THE LIPOLYTIC ACTIVITY OF ANTITHROMBIN III. M.Nakagawa, S.Okuda, M.Watada, and H.Ijichi. Kyoto Prefectural University of Medicine, Kyoto, Japan.

Antithrombin III in the circulating blood is known to play an important role on the coagulation mechanism of the blood and is reported to be decreased in its amount on the cases of myocardial infarction and arteriosclerosis, in the meanwhile the arteriosclerosis has been considered to be one of thrombogenic factors. This research was designed to investigate the effects of antithrombin III on the relationship between fibrin formation and atherosclerosis.

Antithrombin III was purified from human defibrinated plasma by Heparin Sepharose Affinity Chromatography. Using this antithrombin III as an enzyme, its lipolytic activity was assayed spectrophotometrically detecting the produced free fatty acid from various substrates, Intralipid, Triolein, and Human Chylomicron respectively. Lipoprotein lipase activity was detected in the purified antithrombin III. Intralipid was hydrolyzed to a greater extent than triolein or chylomicron and it is concluded that antithrombin III in the circulating blood has not only the inhibitory activity of thrombin but also has the lipolytic activity. From these observations it is considered that this lipolytic activity is beneficial for preventing the developement of atherosclerosis and also of thrombosis.

CHANGES IN THERMODYNAMIC PARAMETERS IN THE INHIBITION OF THROMBIN BY BOVINE ANTITHROMBIN III. Robert H. Yue, Toby Starr and Menard M. Gertler. Institute of Rehabilitation Medicine, New York University Medical Center, New York, New York, U.S.A.

The inhibition of thrombin by isolated bovine antithrombin III (1,200 units/mg of protein) was studied under a variety of experimental conditions. This inactivation reaction follows a second-order reaction and the rate constant depends on a number of parameters. The second-order rate constant decreases with an increase of thrombin concentration. However, at a constant initial concentration of thrombin, the measured amount of antithrombin III present in a sample is directly proportional to the aliquot of the sample. The rate of inactivation was investigated with changes of temperature. For example, at an initial thrombin concentration of 6.8 units/ml and antithrombin III concentration of 4.2 units/ml, the reaction proceeds with E_a of 25 Kcal/mole and $\Delta S^{\ddagger}$ of 42 e.u./mole. In the presence of 0.0015 unit of heparin/ml and with similar concentrations of thrombin and antithrombin III, the reaction proceeds with E_a of 16 Kcal/mole and $\Delta S^{\ddagger}$ of 14 e.u./mole. Therefore, the presence of heparin causes a lowering of the activation energy and a concomitant decrease in the entropy of activation. This change in the thermodynamic parameters allows the inactivation reaction to proceed much faster in the presence of heparin. The effect of heparin may be the result of solvent macromolecular interaction in this inhibition reaction.