

METABOLISM OF I-131 ANTITHROMBIN III IN DOGS. S.H. Wentland, P.S. Damus, B.D. Leonard, A.A. Zamzam, and E.B. Reeve. Univ. Colo. Med. Ctr., and UCLA.

The turnover of canine antithrombin III (AT3) was studied in dogs as a probe of the generation and removal of proteases (e.g. thrombin). AT3 was prepared from dog plasma by affinity chromatography using heparin Sepharose. It was homogeneous by polyacrylamide gel electrophoresis, and showed one major and one minor band by isoelectric focussing. The specific activity (relative units/O.D.) was 1400-fold that of the starting defibrinated plasma. Under suitable conditions labelling efficiency was 80%, ca. 1.2 gram atoms iodine per mole AT3 were incorporated, and specific activity of the labelled AT3 averaged 1200 relative units/O.D. Turnover studies were made by measuring both plasma and whole body counts over 15 days. Later decay of whole body counts was exponential and paralleled that of plasma counts indicating the presence of a single labelled protein degraded at a constant rate. Less active preparations labelled with I-131 were found to have longer half-lives than this preparation and slopes that became flatter with time, indicating contaminating slower metabolizing components. I-131-AT3 turnover studies in dogs that were sedated (to facilitate whole body counting) did not differ significantly from those in untreated dogs. Analysis of fractions obtained from plasma some days after I-131-AT3 injection suggests formation of higher molecular weight metabolites. Some of these are comparable to complexes of AT3 and bovine thrombin formed *in vitro*. Thus, we have prepared a highly active, labelled AT3, demonstrated its suitability for use in turnover studies and have obtained preliminary observations concerning thrombin turnover. (Supported in part by USPHS NIH HL02262; Colorado Heart Assoc.)

DETECTION OF ANTITHROMBIN III /AT-III/ COMPLEXES IN "HYPERCOAGULABLE" AND HYPERFIBRINOLYTIC STATES. G. Sas, Á. Köves and I. Pető. Postgraduate Medical School, Budapest, Hungary.

As it was previously described, the complex of thrombin-AT-III and "free" /uncomplexed/ AT-III can be discerned by a modified crossed immunoelectrophoresis method /heparin in agarose/. Further investigations with the same technique showed that activated factor X /Xa/ and plasmin also form complexes with purified AT-III which, just as thrombin-AT-III complex, migrate slower, than "free" AT-III. On the basis of these observations we assumed a significant increase of the quantity of these AT-III fractions in various "hypercoagulable" and hyperfibrinolytic states, mainly in DIC.

Only about one third of patients with chronic DIC displayed significant increase of AT-III complexes in their plasmas. In a few patients with deep venous thromboses a similar increase of complexed AT-III was observed. In cirrhotic patients with low prothrombin level the administered PPSB concentrate brought about a mild DIC with a striking decrease of "free" AT-III concentration but there was no increase of the complexes. Streptokinase therapy did not increase the quantity of the slow moving fractions of AT-III at all, but the concentration of "free" AT-III decreased significantly.

A fast *in vivo* clearing mechanism of the AT-III complexes can be postulated which could account for the absence of a significant increase of AT-III complexes while the "free" AT-III concentration decreases.

ANTITHROMBIN III RESPONSES IN DOGS TO VARIOUS STIMULI. Y. Takeda and N. Kobayashi. University of Colorado Medical Center, Denver, Colorado, U.S.A.

*In vivo* responses of antithrombin III (AT) to various stimuli were studied to characterize the AT system, using I-125-labeled AT (I-125-AT) as a tracer. Five dogs were used for each study and the radial immunodiffusion was used to measure plasma AT concentration. A single intramuscular injection of 20 mg estradiol caused a 20% decrease of plasma AT concentration in 6 days without appreciable changes of plasma  $t_{1/2}$  of I-125-AT. A single intramuscular injection of 250 mg of progesterone did not produce any appreciable changes of plasma AT concentration or the plasma  $t_{1/2}$  of I-125-AT. However, intramuscular and intravenous injections of a total of 750 mg of cortisol caused a 17% increase of plasma AT concentration in a day without alterations of plasma  $t_{1/2}$  of I-125-AT. Next, the AT responses to thrombophlebitis produced by a previous method were studied. The results indicated no appreciable changes of plasma AT concentration or of plasma  $t_{1/2}$  of I-125-AT. However, a single intravenous injection of 3 ml of typhoid vaccine showed a 25% decrease of plasma AT concentration in a day with a moderate shortening of plasma  $t_{1/2}$  of I-125-AT. Further studies in heparinized dogs showed similar effects with typhoid vaccine. These results indicate that estradiol causes a decreased rate of AT synthesis, that progesterone has no appreciable effects on AT metabolism, that cortisol increases the rate of AT synthesis, that localized thrombophlebitis has no appreciable effects on AT metabolism and that typhoid vaccine produces an increased catabolic rate of AT by unknown mechanisms other than accelerated coagulation processes.