RADIOIMMUNOASSAY OF THROMBOXANE B_2 . V. Ferraris, J.B. Smith and M.J. Silver. Cardeza Foundation and Dept. of Pharmacology, Thomas Jefferson University, Philadelphia, Pa., 19107.

Thromboxane B_2 (TxB_2) is one of the major end products of the cyclooxygenation of arachidonic acid (AA) in blood platelets, lung and certain other tissues. The immediate precursor of TxB_2 is the unstable thromboxane A_2 which has been reported to cause platelet aggregation and to constrict coronary arteries as well as the respiratory tree. We report here on the development of a novel radioimmunoassay for TxB_2 in human plasma. Radioactive TxB_2 was prepared by incubating 3H -AA $_3$ (sp. act. = 64 Ci/mM) with a suspension of washed rabbit platelets for 10 min. at 37°C. The 3H - TxB_2 in the lipid extract was subsequently purified by silicic acid column chromatography. Antibodies to TxB_2 were generated by immunizing rabbits with TxB_2 which had been coupled to albumin using 1,1'-carbonyldiimidazole. The binding of 3H - TxB_2 to diluted plasma (final dilution = 1/1500) from immunized rabbits was inhibited by authentic TxB_2 (Nelson et al, Tetrahedron Letters No. 37, 1976) but not by prostaglandins. Thus, 1.5 picomoles of cold TxB_2 caused 50% inhibition of the binding of 3H - TxB_2 while no cross reactivity was observed with PGD_2 , PGE_2 or $PGF_{2\alpha}$. Small amounts of TxB_2 (10–50 nM) were found in normal human plasma but large amounts (~500 nM) were found in sera obtained from normal human blood clotted at 37°C. The assay should be useful for determining plasma levels of TxB_2 in various disease states. The work supported in part by Grant HL-14890 from the NIH. V.F. was funded by the U.S. Army (AR601-112).

POSTER SYMPOSIUM II

Fibrinolysis: Chromogenic Substrate Assays.

AMPLIFICATION OF COLOR YIELD OBTAINED USING A CHROMOGENIC SUBSTRATE FOR DETERMINING PLASMINOGEN ACTIVATOR. Robert B. Friedman, Hau C. Kwaan and Marija Szczecinski. Northwestern University Medical School and Veterans Administration Lakeside Hospital, Chicago, IL.

A synthetic chromogenic substrate has recently been reported for use in the specific measurement of urokinase. This material (Chromozym UK, marketed by Pen'pharm, Ltd., Basle) is a synthetic tripeptide linked through an amide bond to the chromogen para-nitroaniline (PNA). The release of free PNA can be monitored spectrophotometrically by measuring the increase of absorbance of light in the range of 380 to 410 nm. The present communication describes our efforts at enhancing the color yield of the assay as well as changing the absorption maximum to a wavelength which can more readily and accurately be measured on inexpensive laboratory spectrophotometers.

Bratton and Marshall have developed a method which can be used for the determination of aromatic amines in biological systems. We have modified this method for use in semimicro applications and have adapted it to the measurement of free PNA in solution. After acidification of an aliquot of the enzyme digest with trichloroacetic acid (TCA), the liberated PNA is diazotized with sodium nitrite. Excess nitrous acid is removed with ammonium sulfamate, and the diazotized PNA is reacted with 1-naphthylethylenediamine reagent. The product has a magenta-red color with an absorption maximum of 545 nm. The substrate is not affected by exposure to TCA, and when fresh reagents are used, a low background is obtained. The color yield during actual enzyme assay is amplified twenty fold. Other coupling agents are also currently under investigation.