

EFFECT OF THROMBIN ON PLATELET ADHERENCE TO CULTURED VASCULAR CELLS: MODIFICATION BY DAPA. R.L. Czervionke, J.C. Hoak, D.L. Haycraft, and G.L. Fry. Division of Hematology-Oncology, Department of Medicine and the Cardiovascular Center, University of Iowa College of Medicine, Iowa City, IA.

The role of thrombin in inducing adherence of platelets to cultured vascular cells is complex and may involve factors of both platelet and vascular origin. Dansylarginine N-(3-ethyl-1,5-pentanediyl)amide, DAPA, a fast-acting inhibitor of thrombin, was used in this study to evaluate platelet activation and endothelial PGI<sub>2</sub> release induced by thrombin. Monolayers of endothelial cells and fibroblasts were cultured from human umbilical cord vessels. Empty culture dishes were used as a control. Some vascular cells were treated with aspirin (ASA) to inhibit PGI<sub>2</sub> formation before they were incubated with <sup>51</sup>Cr-platelets for platelet adherence studies. PGI<sub>2</sub> was assayed by radioimmunoassay for 6-keto-PGF<sub>1α</sub>. In one study, endothelium was incubated with 0.3 U thrombin for 2 min. before 10 μM DAPA was added. Platelets were next mixed with this solution and adherence was determined. DAPA caused a decrease in thrombin-induced platelet adherence to normal endothelium from 8% to 2%. When ASA-treated endothelium was employed, DAPA decreased adherence from 59% to 2%. In another study, <sup>51</sup>Cr-platelets were first aggregated with 0.1 U thrombin, then 1.3 μM DAPA was added, and these aggregates were incubated with the monolayers. Similar experiments without DAPA served as positive controls. DAPA did not reverse the aggregate formation, but it did reduce platelet adherence to normal endothelium from 38% to 1%, and to ASA-treated endothelium from 68% to 11%. In contrast, DAPA had little effect with fibroblasts (81% to 71%), ASA-treated fibroblasts (82% to 67%), and the empty dish control (86% to 66%). These results suggest that thrombin-induced platelet adherence in this system involves more than just an effect upon platelets. In addition, they provide further evidence that the non-thrombogenic nature of endothelium persists despite the absence of PGI<sub>2</sub>.

## 0217

LOCALIZATION OF ANTITHROMBIN III ON THE VASCULAR ENDOTHELIUM AND ITS FUNCTION. M.Nakagawa, Y.Okajima, T.Kitani, M.Watada, H.Yoshikawa, R.Hino, H.Ijichi. Second Department of Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Vessel wall is known to be rich in sulfated glycosaminoglycans (GAGs) including heparin and they may serve as the natural catalysts of antithrombin III (AT III). This paper reports the possible role of AT III on the development of anticoagulant activity of activated AT III on the vessel wall. The freshly prepared rat aortic ring was washed in 0.05M Tris HCl 0.15M NaCl buffer (pH 7.5) with or without protamine, and was fixed for histochemical and immunofluorescent examinations. Anti-rat AT III serum was obtained from rabbit immunized with the purified AT III. Mucopolysaccharide stainings including Alcian Blue revealed the sulfated GAGs on the endothelium and the media. The GAGs on the endothelium corresponded with the specific AT III fluorescence localization. Protamine treatment disclosed the disappearance of the AT III fluorescence on the surface of endothelium. AT III-heparin complex was observed to be dissociated by protamine treatment through the investigations with affinity chromatography and sephadex G75 column chromatography, and the separated AT III was proved to recover the original progressive antithrombin activity.

Based on these experimental results it is concluded that the localized AT III on the surface of endothelium may be present in complexed with the exposed heparin or other GAGs and be present in activated, and that the complexed AT III is considered to inhibit proteases in situ in the circulating blood to prevent the activation of coagulation process. This phenomenon may have a clinical significance on the pathogenesis and treatment of thromboembolic vascular diseases.

## 0216

REGULATION OF ADENYLATE CYCLASE-PHOSPHODIESTERASE BY TESTOSTERONE IN CULTURED ENDOTHELIUM. S.S. Ahmed and G.J. Stewart. Thrombosis Research Center, Temple University Medical School, Philadelphia, PA 19140.

The effects of testosterone on the activities of the enzymes involved in cyclic adenosine 3':5'-monophosphate (cyclic AMP) metabolism in primary bovine endothelial cell cultures were examined. Adenylate cyclase was measured under basal and 10 mM NaF stimulated conditions. In control cultures, the enzyme activity remained essentially unchanged for 48 hours and then showed a significant increase ( $p < 0.05$ ) through 96 hours. Addition of testosterone ( $1 \times 10^{-7}$  M) six hours after plating stimulated the enzyme activity causing a significant increase ( $p < 0.05$ ) at 72 hours of incubation. Addition of 10 mM NaF enhanced basal activity as well as testosterone stimulated activity of adenylate cyclase throughout the experimental period. Low and high affinity forms of cAMP phosphodiesterase were measured independently by varying the substrate concentration. At 250 μM substrate the low Km enzyme contributed very little (about 8%) to the activity whereas at 0.1 μM substrate the contribution of the high Km form was about 26%. The activity of both forms fell rapidly during growth phase of control endothelial cells. The addition of  $1 \times 10^{-7}$  M testosterone at the time of plating caused little change from the control level for the first 48 hours, i.e. the activity of phosphodiesterase fell at the same rate in control and treated cultures. Further incubation resulted in marked inhibition of both forms of phosphodiesterase by testosterone. Testosterone added to subconfluent cultures at low concentration ( $1 \times 10^{-9}$  M) failed to stimulate adenylate cyclase but did inhibit low Km phosphodiesterase ( $p < 0.05$ ). A high concentration ( $1 \times 10^{-7}$  M) of testosterone stimulated adenylate cyclase activity and markedly decreased activities of high and low Km phosphodiesterase. This study showed that testosterone induced a major change in cyclic nucleotide metabolism, an important factor in regulating cell function (cell adhesion in this case).

## 0218

POSTPARTUM CYTOTOXIC ENDOTHELIAL ANTIBODIES. T. Parsons, J. Hoak, C. Schroeder, N. Goeken, and J. Thompson. Dept. of Medicine, University of Iowa and Veterans Administration Hospitals, Iowa City, IA.

HLA and non-HLA antigens have been identified on human endothelial cells (HEC). A double fluorochromatic assay was used to detect specific and nonspecific cytotoxic antibodies to endothelial antigens in sera from postpartum women and in patients with thrombotic disease. Single donor umbilical vein HEC were placed in histocompatibility typing wells, and incubated overnight at 37°C, 4% CO<sub>2</sub> atmosphere, to form a monolayer. Five μl of test serum was added to each monolayer and incubated for 1 hr. at 37°C. Serum was removed and five μl non-toxic rabbit complement was incubated with the HEC for 2 hrs. at 37°C. Staining was then performed with diacetylfluorescein and ethidium bromide, and the extent of cytotoxicity was expressed as per cent cell kill. Fifty-six consecutive consenting mothers had 72 hour postpartum sera screened. Cytotoxic antibody to endothelium was present in 10. Three of the 10 sera scored greater than 80 per cent kill with over 80 per cent of the HEC monolayers. Four other postpartum sera that had exhibited HEC cytotoxicity were studied. These 7 sera shared additional characteristics: (1) all showed 60 to 100% reactivity with standard lymphocytotoxicity testing, (2) all failed to induce platelet aggregation, and (3) complement-mediated HEC toxicity was removed by adsorption of the sera with pooled platelets in 6 of 7. The clinical relevance of this cytotoxic HEC activity has not been delineated but an association was found in a 28 yr. old, twice pregnant woman with recurrent deep vein thrombosis and pulmonary embolism. Her anti-endothelial antibody characteristics were like those found in the 7 postpartum women. These results suggest a potential relationship between endothelial injury and thromboembolic complications.