

SYNTHESIS OF PROCOLLAGEN BY ENDOTHELIAL AND SMOOTH MUSCLE CELLS *IN VITRO*. E. Macarak and N. A. Kefalides, University of Pennsylvania, Departments of Medicine and Biochemistry and Biophysics, Philadelphia, Pa., U.S.A.

The synthesis of procollagen by either calf endothelial cells or smooth muscle cells in culture has been studied. Endothelial cells were characterized by the presence of factor VIII antigen and factor VIII-related antigen. Endothelial or smooth muscle cells incorporated [^{14}C]proline into non-dialyzable protein and synthesized hydroxy[^{14}C]proline. Gel filtration (Agarose A5m-SDS) indicated major differences in the molecular properties of the [^{14}C]procollagen and [^{14}C]non-collagen proteins synthesized by these cells. Endothelial cells synthesize a procollagen component which, after reduction, contains approximately 50% of the hydroxy[^{14}C]proline synthesized and elutes near the exclusion volume. This fraction contains 14% of the total hydroxy[^{14}C]proline as the 3-isomer. Two additional components eluted with apparent mol. wts. of 250,000 and 140,000. Only the 140,000 mol. wt. component contains hydroxy[^{14}C]proline. Gel filtration (Agarose A5m-SDS) of the [^{14}C]protein synthesized by smooth muscle cells showed only a single component eluting with an apparent mol. wt. of 130,000. The percent hydroxylation of proline in the peak fraction was 44. The percent of 3-hydroxy[^{14}C]proline was 2%. These data indicate that endothelial cells and smooth muscle cells in culture most likely synthesize different kinds of procollagen (Supported by USPHS Grant AM-14526, HL-18827 and HL-15061).

CULTURED NORMAL AND von WILLEBRAND PORCINE AORTIC ENDOTHELIAL CELLS-AN *IN VITRO* MODEL FOR STUDYING THE NATURE AND SITE OF PLATELET-ENDOTHELIAL COMPONENT INTERACTION. F.M. Booyse, D.N. Fass and E.J.W. Bowie. Rush University, Chicago, Ill., U.S.A. and Mayo Clinic, Rochester, Mn., U.S.A.

Normal and von Willebrand(vWd) porcine aortic endothelial cells(EC) have been maintained and subcultured for 10-12 months(14-18 passages) without any apparent change in characteristic growth morphology or ultrastructure. Immunofluorescence staining of these EC for ristocetin-Willebrand factor(RWF), using monospecific rabbit anti-porcine RWF(arWF), showed differences in the extent and nature of intercellular staining and the apparent absence of extracellular EC associated RWF-containing material(filaments) in vWd cells. Platelet-endothelial(damaged) interaction was decreased in normal EC cultures by pretreatment of the cultures with arWF, no platelet interaction was seen with untreated vWd EC. These cultures were used as an *in vitro* model system(optimal pH, divalent cations, protein, exposure time and rotation speed) for studying the nature, extent and differences in the platelet-microfilament interaction in normal and vWd EC. Preliminary data on the microfilament site of attachment of washed platelets or antibody-coupled beads, the interaction of platelets with matrix-bound purified RWF and the presence of immunologically identifiable RWF-containing filaments in normal and vWd EC suggest a possible role for endothelial-bound RWF in platelet-vessel wall interaction.

STIMULATION OF CULTURED ENDOTHELIAL CELL PROLIFERATION AND METABOLISM BY PLATELETS AND VASO-ACTIVE AGONISTS. D. Shepro and P.D'Amore. Boston University, Departments of Biology and Surgery, Boston, Massachusetts, U.S.A.

Platelets via their secretions communicate and regulate endothelial cells (EC), which function as first level sensors in a biofeedback mechanism to maintain blood and tissue fluid homeostasis. Platelets at a concentration of $10^7/\text{mm}^3$, serotonin (10^{-5}M), thrombin (1 unit/ml), ADP (10^{-6}M), catecholamines (10^{-6}M) and histamine (10^{-6}M) stimulate EC proliferation in culture from 150-1000 percent of controls. That agonists so diverse in form have a similar effect suggests the mobilization of a second messenger, such as calcium, as the common denominator.

^{45}Ca influx in cultured EC increased 5-24 times that of controls following incubation with platelets, platelet secretions, and substances known to circulate in blood following vascular injury. Increased adenine uptake was also observed in response to these agonists, which supports the premise of a regulating role for platelet secretions on EC. Lastly, the increased calcium flux was correlated with the induction of ornithine decarboxylase (ODC) activity. ODC is the rate limiting enzyme in the synthesis of polyamines that are theorized to regulate many cellular functions. ODC induction was 15 fold above baseline following stimulation with complete media, peaking at 3 hr. Preincubation with lanthanum chloride (LaCl_3) inhibited this stimulation; the inhibition was reversed by the removal of the LaCl_3 . A similar response was obtained with 2 mM ethylene glycol-bis N,N'-tetraacetic acid in the media. These data support the validity of using EC in culture to simulate *in vivo* conditions, and the measurement of calcium flux, adenine uptake, and ODC activity, or combinations of the three, are significant parameters when cultured EC are used as an assay system.