

CONTROLLED STUDIES ON THE PATHOGENESIS OF MICROVASCULAR ENDOTHELIAL DAMAGE IN HYPERACUTE CARDIAC ALLOGRAFT REJECTION. R.D.C. Forbes, R.D. Guttman. Department of Pathology, Transplantation Service, Royal Victoria Hospital and McGill University, Montreal, Canada.

Widespread platelet aggregation and microvascular endothelial damage are characteristic features of the early posttransplant phase of hyperacute rat cardiac allograft rejection. Vascular staining for rat C3 and rat IgG are consistent immunohistochemical correlates of these alterations. In order to determine the role of platelets and of complement in the pathogenesis of microvascular destruction in hyperacute rejection, a series of ACI rat cardiac allografts and LEWIS (LEW) syngeneic heart grafts transplanted to ACI skin presensitized LEW recipients were studied over the initial 15 minutes posttransplantation. Intravenously administered colloidal carbon was utilized as a tracer for detection of vascular damage by combined light and electron microscopy. All allografts in unmodified combinations showed extensive platelet aggregation and microvascular endothelial damage. Allografts transplanted to recipients in which C3 had been effectively depleted by cobra venom factor showed no microvascular alterations and were comparable to syngeneic controls. There was no depression of recipient platelet counts in the C3 depleted group at the time of transplantation. Allografts transplanted to recipients in which a profound thrombocytopenia had been induced as a result of prior administration of a rabbit antirat platelet globulin preparation showed extensive microvascular destruction at 15 minutes posttransplantation. Recipient platelet depleted syngeneic heart grafts showed no vascular alterations and were comparable to unmodified syngeneic controls. There was no depression of serum hemolytic C3 in platelet depleted recipients at the time of heart graft placement. This study indicates that complement activated by donor specific alloantibodies may directly induce endothelial damage in hyperacute rat cardiac allograft rejection and that platelets are not critical initiators of this process.

## 0633

10:30 h

VASCULAR SMOOTH MUSCLE CELL PROLIFERATION CAUSED BY A REMOTE INJURY. M.B. Stemberman, R.T. Gardner and R. Fuhro. Department of Medicine, Division of Thrombosis and Hemostasis, Beth Israel Hospital and Harvard Medical School, Boston, MA.

Proliferation of vascular smooth muscle cells (SMC) has been postulated to occur from blood-borne mitogenic stimuli. The hypothesis we tested states that SMC's can be stimulated to enter DNA synthesis and proliferate by a humoral material(s) released from a remote site. Rabbits aortas were balloon de-endothelialized in the following manner: Animals were ballooned throughout their aortas at day 0 except Group IV; Group I were not re-injured thereafter, Group II, only the abdominal aortas were re-ballooned at day 4, Group III were sham-operated (femoral artery tied-off) day 4, and Group IV underwent de-endothelialization of only the abdominal aortas on day 0 and 4. Animals were sacrificed by exsanguination on days 4, 4.5, 4.75, 5, 6, 7, 14 and 28. One hour prior to sacrifice, each animal received 0.5  $\mu$ Ci/gm of  $^3$ H-Thymidine I.V. and 1/2 hr later 5 ml of Evans Blue dye. Within one minute following death, the thoracic segment (3rd to 6th intercostal arteries) was removed and processed for SMC DNA specific activity (SA). Adjacent thoracic segments as well as abdominal aortas were immersed in 2.5% glutaraldehyde and processed for morphology and morphometry. Group II SMC SA showed a sharp rise beginning at day 4.5 and peaked at day 5 ( $335 \pm 62$ ) returning to control levels by day 7. Comparison of Group II with Groups I and III respectively at day 5 ( $86 \pm 19$ ,  $42 \pm 8$ ) were significantly different  $p < .01$ . Group IV animals showed no rise in SA. Intimal cell nuclei per 0.1 mm internal elastic lamina, counted at day 7, showed significant differences also ( $p < .01$ ); Group II  $9 \pm 0.7$  Group I  $5 \pm 0.3$ . These data demonstrate that rabbit aortic SMC's can be caused to proliferate by a remote vascular injury likely due to a humoral substance(s) perhaps released from platelets. Thus, direct vascular injury with release of potential mitogens into the vessel wall underlying the injured tissue is not a prerequisite of SMC proliferation and myointimal growth.

## 0632

10:15 h

DAMAGE TO ENDOTHELIAL CELLS IN THE EAR ARTERY OF RABBITS ON AN ATHEROGENIC DIET. M.J. Silver, A.W. Sedar, C.M. Ingerman-Wojenski, M. Nissenbaum, D. Klerfeld, and D.M. Kritchevsky. Thomas Jefferson University and University of Pennsylvania, Philadelphia, PA 19107 U.S.A.

Male New Zealand rabbits were fed a diet containing 2% cholesterol, 6% peanut oil and 92% Purina Chow for periods between 6 and 9 weeks. Examination of the inner surface of the central ear artery by scanning electron microscopy (after perfusion in situ with Tyrode's solution followed by 1% glutaraldehyde) revealed damage to the endothelium which appeared to be considerably worse at 9 weeks than at 6 weeks. At six weeks the damage included irregularly shaped cells, some breaks at intercellular junctions and occasional holes in the cells. At 9 weeks more severe damage was seen. This included many misshapen cells, cells with holes, and many cells lifting off from the vessel wall and beginning to expose sub-endothelial tissue. In 3 rabbits (respectively on the diet for 6, 7 or 9 weeks) arrays of dark "spots" were seen in many cells. Such "spots" were never seen in endothelial cells of normal rabbits. After 1 ml of 0.83 mM sodium arachidonate (AA), instead of Tyrode's solution, had been perfused through the arteries, further damage was seen. This damage included the lifting off of many cells and formation of many holes. Effluent from the arteries perfused with AA contained 6-keto-PGF $_{1\alpha}$  and TXB $_2$  (measured by specific radioimmunoassays) in amounts similar to those produced by arteries of rabbits fed a normal diet. These metabolites were not detected in effluents of arteries perfused with Tyrode's solution.

This report shows that an atherogenic diet may cause early morphological changes in a peripheral artery of the rabbit and suggests that widespread damage to arterial endothelium may result from atherogenic diets. Apparently, this damage does not impair the ability of the vessel wall to convert AA into prostacyclin. The rabbit ear artery system appears to be a simpler way to study the early changes in arterial endothelium during experimental atherosclerosis than studies on the aorta.

## 0634

10:45 h

THROMBIN INDUCED CONTRACTION OF ISOLATED SMOOTH MUSCLES. Jawed Fareed, Harry L. Messmore, G. Kindel, A. G. Karczmur, and J. W. Fenton II. Loyola University Medical Center, Maywood, Illinois 60153, U.S.A. and Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201, U.S.A.

During the clotting of whole human blood or plasma, we have observed the generation of vasospasmodic activity with isolated smooth muscle preparations. Since kallikreins reportedly have musculotropic effects, such effects of related serine proteases were examined on isolated guinea pig ileum and rat uterus smooth muscle preparations. Like glandular kallikrein, human  $\alpha$ -thrombin caused pronounced muscular contractions of these preparations and did so more effectively than trypsin and much more so than plasmin, activated coagulation factor X or XII, or noncoagulant  $\beta$ - and  $\gamma$ -thrombins. Levels as low as 0.1 to 1 clotting (NIH) units/ml final bath concentration of  $\alpha$ -thrombin caused such contractions (5 units/ml grossly contracted muscle preparations) and the contractile activity was inhibited by either hirudin or antithrombin III plus heparin, indicating the requirement for the catalytically active enzyme. These contractions were not blocked by antihistaminic (tripellamine), antimuscarinic (atropine), or antiganglionic (mecamylamine) agents, further suggesting that the contractions were not mediated by an autonomic mechanism but rather were attributable to a direct function of the enzyme. Because physiologic concentrations of less than 5 units/ml were effective, our data imply that vasospasmodic responses may not only result from thromboxanes and platelet release products (e.g. serotonin and ADP) but also to direct  $\alpha$ -thrombin induced contractile effects on smooth muscles exposed at sites of vascular injury. Such smooth muscle contractions may facilitate in the prevention of blood loss or may contribute to pathologic states (e.g. ischemia, stroke) impeding blood circulation.