

**INJURY OF ENDOTHELIAL CELLS IN CULTURE INDUCED BY LOW DENSITY LIPOPROTEINS.** T. Henriksen, S.A. Evensen and B. Carlander. Institute for Surgical Research and Medical Department A, Rikshospitalet, Norway.

The effect of low density lipoproteins on human endothelial cells in primary culture was investigated. The effect was evaluated by use of a  $^{51}\text{Cr}$ -release assay and phase contrast microscopy. When the cells were exposed to low density lipoproteins (LDL,  $d=1.019-1.063\text{g/ml}$ ) suspended in RPMI 1640 the endothelial cells underwent extensive morphological changes and detached from the bottom of the culture vessel. Addition of lipoprotein-poor serum ( $d>1.21\text{ g/ml}$ ) prevented the injurious effect of the LDL fraction at low concentrations, and to a lesser extent as the LDL concentration was raised. Unfractionated serum from hyperlipoproteinemic patients (type IIa) did not appear to injure endothelial cells. Addition of the LDL fraction, however, had a more pronounced effect on the cells when added to hyper-lipoproteinemic serum than to normal human serum. Thus, LDL as obtained by ultracentrifugation may have an injurious effect on cultured human endothelial cells. Secondly, hyperlipoproteinemic serum appears to contain factors which augment the effect of the added LDL fraction.

**THE EFFECT OF PROSTAGLANDINS  $G_2$ ,  $D_2$ ,  $E_2$ ,  $E_1$  AND ARACHIDONIC ACID ON THROMBUS FORMATION IN VIVO.** J. Westwick and G.P. Lewis. Royal College of Surgeons of England, London, England.

Arachidonic acid (AA) and prostaglandin (PG)  $G_2$  have been shown to be precursors of both pro-aggregatory and anti-aggregatory agents *in vitro*. If  $PGG_2$  is produced in thrombotic and inflammatory situations, it is important to know its effects on thrombus formation *in vivo*. Mural thrombus formation was induced in the arterioles ( $40-70\ \mu\text{m}$ ) of the hamster cheek pouch by combining micro-electrical damage with perivascular application of ADP ( $10^{-6}\text{M}$ ).

PG or vehicle was applied perivascularly, followed 30 sec and 1 min later by electrical micro-damage and application of ADP ( $10^{-6}\text{M}$ ). The vessel was observed and thrombus formation was quantitated by timing the adherence of thrombi for the following 10 min. Each animal served as its own control and results were expressed as % difference (mean  $\pm$  s.e.) from control.  $PGG_2$ , AA and  $PGE_1$  produced a dose-related (12.5 - 1250 ng) inhibition ( $10 \pm 8\%$  -  $90 \pm 15\%$ ) of thrombus formation.

Both  $PGG_2$  (Lewis, Westwick & Williams, Br.J.Pharmac., 1977, in press) and AA induce a short-lasting vasoconstriction followed by vasodilatation. However, another potent vasodilator,  $PGE_2$ , in a low dose (125 ng) potentiated ( $49 \pm 20\%$ ) while high doses (1250 ng) produced a weak inhibition ( $15 \pm 10\%$ ) of thrombus formation.  $PGD_2$  had little activity up to a concentration of 1250 ng.

These results demonstrate that AA and  $PGG_2$  can be converted to anti-thrombotic agents *in vivo* when applied perivascularly. Since  $PGD_2$  and  $PGE_2$  were not anti-thrombotic, it is possible that the observed effect was due to generation of prostacyclin.

**DEGRADATION OF SULPHATED GLYCOSAMINOGLYCANS AT THE SURFACE OF CULTURED HUMAN ENDOTHELIAL CELLS BY A PLATELET HEPARITINASE.** C. Busch, B. Glimelius, R. Wastesson and B. Westermark. Uppsala, Sweden.

The non-thrombogenic property of the endothelial cell surface is a prerequisite for maintenance of blood circulation. The nature of this property is poorly understood. Recent advances in culturing techniques of endothelial cells *in vitro* may facilitate studies of the surface biochemistry. Human endothelial cells (EC) isolated from umbilical veins were shown to synthesize and secrete sulphated glycosaminoglycans (GAG). The recent finding of a platelet enzyme capable of degrading heparin sulphate (HS) raised the question: Can platelet lysate or a purified heparitinase detach and degrade endothelial HS? EC cultured in the presence of  $^{35}\text{S}$ -sulphate, produce  $^{35}\text{S}$ -labelled GAG which was isolated from the incubation medium from a cell associated trypsin labile pool and from a cellular pool not liberated by trypsin. After 48 hours of incorporation about 95% of  $^{35}\text{S}$ -GAG was found in the medium fraction, 5% in the trypsin fraction and negligible amounts in the cell fraction. In the trypsin pool ("surface fraction") heparin sulphate comprised about 85%, while the remaining 15% consisted of chondroitin sulphate and/or dermatan sulphate. Incubation of  $^{35}\text{S}$ -labelled EC with platelet lysate or a partially purified preparation of the enzyme from the same source caused a marked release of cell-surface associated HS to the incubation medium as oligosaccharides. These effects could be ascribed to heparitinase activity and may alter the properties of the EC-surface and influence the interaction between these cells on one hand and blood cells or plasma components, e.g., coagulation factors on the other.