

1000

**CONTRACTIONS TO PLATELETS IN AORTAS OF CONTROL AND CHOLESTEROL-FED RABBITS.** T.J. Verbeuren, M.J. Van Diest and A.G. Herman. Division of Pharmacology, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Wilrijk, Belgium.

Atherosclerotic aortas obtained from cholesterol-fed rabbits show a decreased responsiveness to noradrenaline, an increased responsiveness to low concentrations of serotonin and an unaltered responsiveness to prostaglandins. In vitro contractions induced by aggregating platelets are largely due to serotonin liberated during the aggregation. The present study was designed to compare the contractile responses to aggregating platelets in aortas obtained from control and cholesterol-fed rabbits.

Male New Zealand rabbits were fed either a control or a 0.3% cholesterol diet during 16 weeks. Macroscopic and microscopic examination of the luminal surface of the aortas obtained from these animals revealed a substantial amount of fatty streaks in the tissues obtained from the cholesterol-fed rabbits. Segments of the aortic arch of the rabbits were then mounted in organ chambers for isometric tension recording.

In both the control and the atherosclerotic aortas increasing concentrations of platelets evoked contractions; the contractions obtained with the lower concentrations of platelets were significantly greater in the atherosclerotic tissues. The maximal responses and the ED<sub>50</sub>-values were comparable in both groups of blood vessels. No significant differences were observed when platelets obtained from control or hypercholesterolemic rabbits were compared. In the control and the atherosclerotic aortas the thromboxane receptor antagonist BML3505 at  $2 \times 10^{-5}$ M did not significantly affect the contractions to platelets obtained from either control or cholesterol-fed rabbits. The serotonin receptor antagonist ketanserin at  $5 \times 10^{-8}$ M nearly abolished the responses to platelets in both groups of aortas.

These experiments illustrate that (1) the contractions induced by rabbit platelets in control and atherosclerotic aortas are mediated by serotonin and (2) the responses to platelets, as those to serotonin, are augmented in the atherosclerotic preparations.

**RAPID AND STRONG PLATELET REACTIONS AND COAGULATION CAUSED BY INJURY OF PROLIFERATING INTIMAL SMOOTH MUSCLE CELLS.** L. Jørgensen (1,2), A.G. Grøthe (2), H.M. Groves (3) and M. Richardson (3). Institute of Forensic Medicine, University of Oslo, Oslo, Norway (1), Institute of Medical Biology, University of Tromsø, Tromsø, Norway (2) and Department of Pathology, McMaster University, Hamilton, Ont., Canada (3).

We have shown that while one balloon catheter injury to rabbit aortae results in formation of a monolayer of platelets on the de-endothelialized surface, a second identical injury 7 days later gives rise to platelet-fibrin thrombi as observed at 30 minutes after the reinjury. The enhanced thrombogenicity the second time was related to damage of the neointima which had formed during the intervening days. It was unclear whether it was due to damage of the cells or of the interstitium. In order to further explore the mechanisms of the enhanced thrombogenicity following reinjury we observed by SEM and TEM the sequence of reactions before and during the first seconds and minutes after the second injury. The neointima was distributed around and opposite the branch orifices; the remainder of the luminal surface consisted of uncovered subendothelium. Within 10-30 seconds after the reinjury some of the neointimal cells were partly detached, and a few platelets and fibrin-like strands were in contact with them. Within 1-5 minutes many platelets, both single and in groups, with or without fibrin strands, had gathered in association with injured smooth muscle cells. The reinjured subendothelium remained uncovered the first 10-30 seconds; at 1-5 minutes an incomplete monolayer of swollen platelets had formed, but no fibrin was observed. These observations confirm that injury to neointima causes increased thrombogenicity. Since the first platelet reactions and coagulation are associated with injured neointimal cells, it is likely that injury to these cells precipitates these reactions.

## DIET, HAEMOSTASIS AND THROMBOSIS

Wednesday

1002

**THE EFFECT OF TWO CHOLESTEROL-LOWERING AGENTS ON PLATELETS IN PATIENTS WITH HYPERCHOLESTEROLEMIA.** A. Nordøy, T. Simonsen, K. Ytre-Arne, V. Lyngmo and B. Svensson. Dept. of Medicine, University Hospital, Tromsø, Norway

Twenty-one subjects with type IIa hyperlipoproteinemia, receiving dietary treatment were given Synvinolin (MK-733), a HMG-CoA reductase inhibitor, 40 mg or Cholestyramin (Questran) 24 g daily for a period of 12 weeks. Serum lipids, platelet cholesterol, phospholipids and fatty acid composition and platelet function were measured before and after the intake of the two drugs.

Both drugs reduced serum total cholesterol with approximately 50%. No significant changes were observed in platelet lipid concentrations or in the primary bleeding time. Collagen induced aggregation and thromboxane (TXA<sub>2</sub>) production were reduced, whereas thrombin induced aggregation and TXA<sub>2</sub> production were unaffected. This study shows that both a cholesterol synthetase inhibitor and Cholestyramin reduce the total serum cholesterol concentration and also reduce platelet aggregation and thromboxane synthesis without changing the platelet cholesterol content or the cholesterol/phospholipid ratio. The effect on serum lipids and platelet function may indicate a beneficial effect of both drugs on arterial disease in patients with hypercholesterolemia.

1003

**INCREASED FACTOR VII REACTIVITY IN THE RABBIT FOLLOWING DIET-INDUCED HYPERCHOLESTEROLAEMIA.** K.A. Mitropoulos (1), S.J. Walter (2), T.W. Meade (1) and M.P. Esnouf (2). MRC Epidemiology and Medical Care Unit, Northwick Park Hospital, Harrow, Middlesex (1) and Nuffield Department of Clinical Biochemistry, The Radcliffe Infirmary, Oxford (2), U.K.

The association of factor VII coagulant activity (VII<sub>C</sub>) with plasma lipid concentrations has been a consistent feature of a number of studies in man and points to plasma lipoproteins as determinants of VII<sub>C</sub>. To modify plasma lipoprotein concentrations and to study the effect of this on VII<sub>C</sub>, rabbits were fed a 1% cholesterol-supplemented diet. Treatment resulted in a many-fold increase in plasma cholesterol concentration with the major fraction of excess cholesterol associated with the very low and intermediate density lipoprotein fractions. VII<sub>C</sub> was considerably higher in rabbits fed 1% cholesterol-supplemented than in rabbits fed the standard diet. In both groups of rabbits, the direction and extent of variation in VII<sub>C</sub> coincided with variation in cholesterol concentration so that over time there were significant and positive correlations between VII<sub>C</sub> and plasma cholesterol. A method that provides a measure of the total functional factor VII concentration (VII<sub>T</sub>) was also used. This assay involves clotting the plasma in the presence of excess tissue factor and therefore the conversion of all VII to the more reactive two-chain form of the protein (αVII<sub>a</sub>). The concentration of αVII<sub>a</sub> present in the serum was measured from the rate of activation of excess of [sialyl-<sup>3</sup>H]-bovine factor X. By day 10 of treatment, and in all further comparisons VII<sub>T</sub> was only slightly higher in the group of rabbits fed cholesterol-supplemented than in that fed the standard diet. This increase in VII<sub>T</sub> is too small to explain the considerable increase in VII<sub>C</sub> in the hypercholesterolaemic rabbit. We conclude that the increase in VII<sub>C</sub> was due to a higher proportion of αVII<sub>a</sub> in the plasma of hypercholesterolaemic rabbits rather than to an increase in the concentration of the single-chain protein.