

HUMAN PLATELET AGGREGATION IN LAMINAR FLOW: STIMULUS AND SHEAR RATE DEPENDENCE, WITH EFFECTS OF ACETYSALICYLIC ACID. W. Yung and M.M. Frojmovic, Department of Physiology, McGill University, Montreal, Canada.

The shear rate (G) dependence of aggregate formation and stability of platelets in human citrated platelet-rich plasma (PRP) in laminar flow was studied for  $G=0-150 \text{ sec}^{-1}$ , a range encountered in many flow conditions, by injecting adenosine diphosphate (ADP) into PRP within a cone-in-plate device at  $37^\circ\text{C}$ . Macroaggregation was assessed by visual observation and light transmission (%T), as routinely done in aggregometry, while actual percent aggregation (PA) was determined by microscopy of sub-samples from the decrease in single platelet concentration. Control PA  $\leq 8\%$  ( $n=8$ ), independent of G. Low [ADP]  $\approx 0.75-1.5 \mu\text{M}$ , causing reversible aggregation in the aggregometer, gave shape change, and stable visible thrombus formation with maximal PA  $\approx 25\%$  at low  $G \leq 30 \text{ sec}^{-1}$ ; the apparent extent of aggregation ( $\Delta\%T$ ) passed through a maximum at  $G \approx 30 \text{ sec}^{-1}$ , with aggregate disruption occurring at  $G > 30 \text{ sec}^{-1}$ . For high ADP  $\geq 5 \mu\text{M}$ , causing stable maximal aggregation in the aggregometer, the rate of aggregation increased continuously with G, with PA 90% and visible thrombi stable for  $G=15-150 \text{ sec}^{-1}$ . Aspirin ( $200 \mu\text{M}$ ) ( $n=8$ ) or indomethacin ( $10 \mu\text{M}$ ) ( $n=2$ ) - treated platelets activated with high ADP  $\geq 5-7.5 \mu\text{M}$ , exhibited the same stable aggregation at low  $G (\leq 30 \text{ sec}^{-1})$  as found for untreated platelets; higher  $G > 30 \text{ sec}^{-1}$  yielded only reversible aggregation as seen in the aggregometer, with PA only partially inhibited. It seems that (1) at high [ADP] ( $\geq 5 \mu\text{M}$ ), increasing G normally increases the rate of platelet aggregation without causing deaggregation, and (2) for [ADP] up to  $\sim 7.5 \mu\text{M}$ , in the absence of endoperoxide synthetase activation, shear stresses ( $\tau$ ) of only  $\approx 1-3 \text{ dynes cm}^{-2}$  prevent the formation of stable large thrombi though still allowing appreciable PA; sufficiently high ADP ( $10 \mu\text{M}$ ) overcomes these  $\tau$  destabilizing effects.

EFFECTS OF SHORT TIME HIGH SHEAR EXPOSURE UPON PLATELET FUNCTION AND THE COAGULATION SYSTEM UNDER HEPARIN ANTICOAGULATION. L.J. Wurzing, R. Opitz\*, P. Blasberg, K. Bialonski\*\* and H. Schmid-Schönbein. Dept. of Physiology and \* Aerodynamisches Institut, RWTH Aachen, F.R.G.

The fact that high shear activates and damages platelets has been suspected to be a major cause of thromboembolism in artificial internal organs (AIO) or in arterial stenosis. In AIO wall shear stresses well above  $50 \text{ Nm}^{-2}$  have been computed to which blood cells are exposed for times in the order of milliseconds (ms). Unfortunately, the studies on this subject employing defined flow conditions operate with exposure times higher than 10 seconds. The purpose of the present study was to elucidate the effects of high shear exposure for ms upon platelet function (ADP induced platelet aggregation (PA), platelet procoagulant activity (PF-3)) under heparin anticoagulation, which is also used in AIO.

To apply shear rates ranging from  $50 - 220 \text{ Nm}^{-2}$  to heparinized PRP for defined exposure times between  $7 - 700 \text{ ms}$  a flow through Couette-viscometer was employed. Platelet factor 3 (PF-3) availability was estimated by using a modified Stypven time technique. Lactic dehydrogenase (LDH) liberation was taken as a measure for platelet destruction. All steps of the experimental procedure were carried out at  $37^\circ\text{C}$ .

1. Alteration of platelets was found to be dependent upon the magnitude of shear stress as well as its duration.
2. With increasing shear stress and/or time of exposure platelet destruction and PF-3 availability were enhanced and ADP induced aggregation was reduced.
3. Shortening of RVT activated clotting time occurred at much lower shear stresses and/or exposure times than platelet lysis did. Threshold shear stress from which on a significant change in the platelet parameters occurred:  
113 ms: lysis (LDH):  $170 \text{ Nm}^{-2}$ ; PF-3:  $57 \text{ Nm}^{-2}$   
7 ms: lysis:  $220 \text{ Nm}^{-2}$ ; PF-3:  $170 \text{ Nm}^{-2}$ .

From our data we conclude that, in the presence of physiological calcium levels (heparin anticoagulation) shear stresses and exposure times that certainly occur in AIO are able to activate platelets and procoagulant potential of blood.

PROSTACYCLIN RELEASE IN VASCULAR PROSTHESES WITH REGARD TO THE NON-THROMBOGENICITY OF THE NEO-INTIMA. H.-M. Fritsche, Th. Holzmann, P. Wendt, W. Erhardt, P.C. Maurer, G. Blümel. Institute for Experimental Surgery and Department of Vascular Surgery, Technical University of Munich, FRG.

There is good evidence that internal surfacing of vascular prostheses by a non-thrombogenic biological layer is important for satisfactory long-term clinical results. However morphological and histological data obtained so far on the so called neo-intima provide little information about the anti-thrombogenic activity of the newly formed endothelium.

In order to characterize the functional activity of the neo-intima we have developed a device to measure prostacyclin ( $\text{PGI}_2$ ) release from the vascular wall into the lumen. Different types of vascular grafts (velour knitted dacron, P.T.F.E. length 10cm, diameter 4-6mm) were placed in the carotid arteries of sheep. After removal the grafts were cut in three transverse sections (proximal, medial and distal third) and were subsequently perfused with oxygenated buffer in order to restimulate the arachidonic acid metabolism in the newly formed vascular wall. Thereafter the sections were filled with a specific volume of buffer and incubated. In the incubated solution  $\text{PGI}_2$  release was measured using a collagen and ADP induced platelet aggregation model and a 6-Keto-PGF  $1\alpha$  radioimmunoassay.

Within four months postoperatively  $\text{PGI}_2$  release increased especially in the proximal and distal sections of the prostheses up to the normal range, while in the medial sections of some grafts even 6 months postoperatively the  $\text{PGI}_2$  release remained weak. Simultaneous investigations obtained by scanning electron microscopy revealed that there is no clear correlation between the morphology of the newly formed endothelium lining different types of vascular prostheses and the functional activity of the neo-intima. This makes it desirable to characterize the functional activity of the neo-intima with regard to the non-thrombogenicity of the newly formed endothelium. Improvements in newly developed vascular prostheses can thus be quantitatively measured.