

PLENARY I

Coagulation

SHEAR RATE DEPENDENCE OF PLATELET ADHESION TO COLLAGENOUS SURFACES IN WILLEBRAND FACTOR-DEPLETED BLOOD. H. R. Baumgartner, Th. B. Tschopp and D. Meyer, F. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland and Institut de Pathologie Cellulaire, Bicêtre, France.

A platelet function defect similar to that in von Willebrand's disease was produced by adding to human blood an antibody raised in rabbits against human factor VIII/Willebrand factor. The effect of this antibody (F VIII Ab) on platelet adhesion was tested in an annular perfusion chamber. Only a small adhesion defect was observed at a shear rate corresponding to that in large arteries (830 s^{-1}). Since bleeding usually occurs from small vessels, platelet adhesion to collagenous surfaces was investigated at higher shear rates corresponding to those in small vessels. The surfaces were exposed to human blood (15mM citrate) at 1, 2 and $4 \times 10^3 \text{ s}^{-1}$ shear rate for 6, $4\frac{1}{2}$ and 3 min, respectively. Different exposure times were chosen in order to obtain in control perfusions 50 - 70 % coverage of subendothelium with platelets at each shear rate. Platelet adhesion and adhesion-induced aggregation were measured morphometrically. As compared to control, F VIII Ab inhibited adhesion to subendothelium by 15 ± 6 , 32 ± 21 , 40 ± 10 and 90 ± 2 % (mean \pm SE) at 0.83, 1, 2 and $4 \times 10^3 \text{ s}^{-1}$ shear rate, respectively. The corresponding inhibition by F VIII Ab observed on the fibrillar collagen of α -chymotrypsin-digested subendothelium was 62 ± 11 , 75 ± 20 , 99 ± 1 and 100 %. In platelet rich plasma, F VIII Ab abolished Ristocetin-, inhibited collagen- and had no effect on ADP-induced aggregation.

Thus the defect in platelet adhesion to collagenous surfaces observed in Willebrand factor-depleted blood is minimal at low (venous) and maximal at high (small vessel) shear rates.

NEW HYPOTHESIS FOR THE MOLECULAR MECHANISM OF SURFACE-DEPENDENT ACTIVATION OF HAGEMAN FACTOR (FACTOR XII). John H. Griffin, Scripps Clinic & Research Fndn., La Jolla, CA, U.S.A.

The surface-dependent mechanism of activation of highly purified human Hageman factor (HF) was studied using ^3H -DFP uptake as a quantitative active site titrant. HF was treated in various ways and the reaction mixture was exposed to 3 mM ^3H -DFP for 5 min at 37° . Following addition of SDS and removal of free DFP by dialysis, the reaction products were analyzed on SDS gels. In solution, the HF zymogen at 80,000 MW took up 0.015 mol DFP per mol HF. HF bound to kaolin, celite, or ellagic acid with or without high MW kininogen took up the same 0.015 mol DFP per mol HF. However, HF bound to celite or kaolin with high MW kininogen and kallikrein took up 0.9 mol DFP per mol HF into a 28,000 MW fragment of HF. In approximately half of these activated HF molecules, this 28,000 MW fragment was linked by disulfide bonds to a 52,000 MW fragment in a surface-bound 80,000 MW form of activated HF. In clotting assays, DFP did not inhibit kaolin-bound HF unless the surface-bound HF first had been proteolytically activated by kallikrein.

Kinetic studies of the cleavage of ^{125}I -HF by kallikrein or by plasmin in the presence of high MW kininogen showed that kaolin-bound HF was cleaved more than 20 times faster than HF in solution.

These results suggest that binding to kaolin or celite or ellagic acid, classically known as "activating surfaces", does not convert a detectable fraction (< 1%) of the bound HF molecules to active enzymes. Rather, surface-binding makes HF molecules much more susceptible to proteolytic activation in the presence of high MW kininogen, and the reciprocal proteolytic activations of HF and prekallikrein are thus greatly stimulated by "activating surfaces".