

THE EFFECTS OF CHYMOTRYPSIN AND OF ADP ON THE BINDING SITE FOR BOVINE FACTOR VIII ON HUMAN PLATELETS. Edward P. Kirby and David C.B. Mills, Dept. of Biochemistry and the Thrombosis Research Center, Temple University, Philadelphia, PA 19140.

The aggregation of human platelets by bovine Factor VIII (Platelet Agglutinating Factor-PAF) is inhibited by exposure of the cells to ADP or chymotrypsin. We have investigated the mechanism of these effects using washed platelets. The washing procedure was modified from the method of Mustard et al. (Brit. J. Haematol. 22:193, 1972), omitting heparin and using a protein-free Tyrode's solution for the final resuspension. The washed platelets were stable and responded to ADP ( $0.1-1 \mu\text{M}$ ) with a shape change and, if fibrinogen was added, with aggregation. Bovine Factor VIII was purified to >90% homogeneity and was labeled with  $^{125}\text{I}$  (approx. 1 atom/subunit) by the IodoGen procedure, with no loss of activity. Aggregation was measured in the aggregometer in the presence of 7 mM EDTA. Binding was measured after incubation of labeled Factor VIII with washed platelets in the presence of 7 mM EDTA for 5 min at  $37^\circ$  without stirring.

Treatment of washed platelets with chymotrypsin progressively destroyed their ability to bind Factor VIII and to be agglutinated by it. Responsiveness to Factor VIII disappeared before any alteration was detected in the ability of platelets to undergo ADP-induced shape change. Treatment of platelets with ADP, however, inhibited agglutination induced by Factor VIII without affecting the binding of Factor VIII to the platelets. Agglutination by wheat germ agglutinin or phytohemagglutinin was not inhibited by ADP treatment. We conclude that chymotrypsin probably inhibits Factor VIII-induced agglutination by destroying the platelet binding site for Factor VIII, but that ADP must act at a point distal to Factor VIII binding. Agglutination of metabolically intact platelets by Factor VIII may not be a simple process, because ADP can specifically inhibit it without affecting Factor VIII binding or aggregation of the platelets by lectins.

## 0951

REQUIREMENT OF FIBRINOGEN AND CALCIUM FOR INTER-PLATELET BRIDGES IN PLATELET AGGREGATION: A HYPOTHESIS. Elizabeth Kornecki and Stefan Niewiarowski. Thromb. Res. Ctr., Temple Univ. Health Sci. Ctr., Philadelphia, PA 19140.

Fibrinogen and calcium are required for the aggregation of platelets stimulated by ADP or pre-treated with proteolytic enzymes. Specific platelet surface fibrinogen binding sites (receptors) are exposed after platelets are stimulated by ADP or pre-treated with chymotrypsin or pronase. It has previously been shown in our laboratory that an intact, symmetrical fibrinogen molecule is essential for fibrinogen binding and fibrinogen-induced aggregation of both ADP-stimulated and proteolytically-treated platelets. Here we propose that the mechanism by which fibrinogen and calcium aggregate platelets is by forming inter-platelet bridges linking the fibrinogen receptors of adjacent platelets together. In support of this proposition are the following new lines of evidence: 1) The fibrinogen-induced aggregations of ADP-stimulated or proteolytically-treated platelets are inhibited by high concentrations of fibrinogen ( $K_d=2.6$  and  $8.5 \times 10^{-5}\text{M}$ , respectively). The fibrinogen binding sites on adjacent platelets, at these concentrations, would be saturated by fibrinogen and therefore no inter-platelet fibrinogen bridges could be formed to hold the platelets together. 2) ADP-stimulated or chymotrypsin-treated platelets aggregated by fibrinogen are deaggregated by chymotrypsin or pronase and this deaggregation coincides with the loss of  $^{125}\text{I}$ -fibrinogen from the platelet surface. 3) Preincubation of platelets with EDTA results in inhibition of both platelet aggregation and  $^{125}\text{I}$ -fibrinogen binding. Following the aggregations of ADP-stimulated or of chymotrypsin-treated platelets by fibrinogen, the addition of EDTA to the platelet aggregates results in both their deaggregation and their loss of bound  $^{125}\text{I}$ -fibrinogen. Thus it appears that divalent cations, especially calcium, are essential for the formation of fibrinogen-linked platelet aggregates.

## 0950

DETECTION OF HIGH AFFINITY FIBRINOGEN BINDING SITES ON HUMAN PLATELETS STIMULATED BY ADP: COMPARISON OF TWO METHODS OF PLATELET SEPARATION. Stefan Niewiarowski, Thomas A. Morinelli and Elizabeth Kornecki. Thrombosis Research Center Temple University Health Sciences Center, Philadelphia, PA 19140.

Binding of fibrinogen to specific receptors on human platelets exposed by ADP results in platelet aggregation. There are controversial data regarding classes and number of fibrinogen receptors, the values range from one to two classes and 1,000-80,000 receptors per platelet as reported in the literature. We have studied the interaction of fibrinogen with a) platelets washed by differential centrifugation according to Mustard and colleagues (washed platelets - WP) and with b) gel-filtered platelets (GFP). Platelet aggregation was studied with  $100 \mu\text{M}$  ADP and with various concentration of fibrinogen. Maximal velocities of aggregation for WP and GFP were 81 and 47 units per min, respectively, and the  $K_m$  values for fibrinogen calculated from the rate of aggregation were  $0.9 \times 10^{-7}\text{M}$  for WP and  $5.8 \times 10^{-7}\text{M}$  for GFP. The level of platelet fibrinogen released into the suspension from WP and GFP amounted to  $2.4 \mu\text{g}$  and  $15.0 \mu\text{g}$  per  $10^9$  platelets/ml, respectively, as measured by the staphylococcal clumping test. Analysis of  $^{125}\text{I}$ -fibrinogen binding data by the method of Scatchard and Feldman revealed 1,300 high affinity receptors ( $K_d$   $3.2 \times 10^{-8}\text{M}$ ) and 80,000 low affinity receptors ( $K_d$   $5.6 \times 10^{-5}\text{M}$ ) for WP. The binding of  $^{125}\text{I}$ -fibrinogen to GFP was greatly diminished. The number of fibrinogen receptors exposed by ADP on GFP and their binding affinity are under investigation in our laboratory. In conclusion, GFP were less sensitive to fibrinogen than were WP as shown in the aggregation and  $^{125}\text{I}$ -fibrinogen binding studies. It appears that the method of platelet separation is critical for the assessment of fibrinogen binding. Platelet activation and release of intact platelet fibrinogen during gel-filtration may interfere with the detection of high affinity fibrinogen binding sites.

## 0952

REACTIVITY OF HUMAN FIBRINOGEN ADSORBED ON TWO DIFFERENT TYPES OF HEMODIALYSIS MEMBRANES. H.Y.K. Chuang, T.R. Sharpton, S.F. Mohammad, N.C. Sharma and R.G. Mason. Department of Pathology, College of Medicine, University of Utah, Salt Lake City, UT 84132.

Cuprophane and polyacrylonitrile are two common materials used as hemodialysis membranes. Examination by both phase contrast and electron microscopy of both types of material after clinical *ex vivo* use with uremic patients indicated that cellular deposition was extensive on Cuprophane but not on polyacrylonitrile. *In vitro* studies with  $^{125}\text{I}$ -labeled human fibrinogen or immunoglobulin G (IgG) showed that the adsorption of fibrinogen and IgG was greater on polyacrylonitrile than on Cuprophane. These results appear to be in conflict with the general concept that materials that adsorb large quantities of protein, especially fibrinogen and IgG, will promote greater adhesion of platelets and leukocytes. Further studies of fibrinogen adsorbed on polyacrylonitrile surfaces indicated that the adsorbed fibrinogen: (a) was not readily accessible for reaction with  $^{125}\text{I}$ -labeled antifibrinogen IgG, (b) was not desorbed readily from the surface, (c) was not appreciably displaced by other plasma proteins such as albumin, IgG, except fibrinogen, and (d) did not promote the adhesion of  $^{51}\text{Cr}$ -labeled platelets or polymorphonuclear leukocytes. These data indicate that fibrinogen, though present in high concentration on polyacrylonitrile, may adsorb in a biologically inactive form. This could be due to either the direct involvement of fibrinogen active sites in the interaction of the protein molecule with polyacrylonitrile or a conformational change in the fibrinogen molecule upon adsorption. Our observations suggest that the thrombogenicity of an artificial surface may not be assessed by the type and amount of various protein adsorbed but is likely determined by the reactivity of specific adsorbed protein species.