THE ROLE OF FIBRINOGEN IN THE INTERACTION OF PLATELETS WITH PARTICLES. Sharron L. Pfueller and B.G. Firkin. Department of Medicine, Monash University, Alfred Hospital, Melbourne, Australia.

The initial interaction of polystyrene latex particles with platelets has been studied by means of their agglutination with platelets measured nephelometrically. 2 to 10 min. after addition of latex particles to citrated PRP at 37°C slow agglutination occurs. When latex is preincubated with PPP for 5 to 40 mins at 37°C, sedimented and resuspended in buffer it is "activated" and produces an immediate and rapid agglutination when added to PRP. This "activation" like activation of zymosan particles (Z) occurs in plasma deficient in Factor VIII and/or von Willebrand factor, is not prevented by hirudin, and does not occur in plasma heated at 56°C for 10 min or in recalcified PPP; addition of fibrinogen restores activity to these treated plasmas. Unlike Z activation, it is not inhibited by heparin, EDTA, or destruction of Complement. Activation can be mimicked by preincubation with fibrinogen alone, but not with IgG or albumin. In PRP, in contrast to washed platelets, latex preincubated with IgG caused less agglutination than when pretreated with fibrinogen or buffer. Agglutination by activated latex in PRP, unlike aggregation by activated Z and other aggregating agents, causes only slight ^{14}C-serotonin release and is not inhibited by aspirin, indomethacin or adenosine,but, like the reaction with Z, is inhibited by EDTA, chlorpromazine, cocaine-HCl, N-ethylmaleimide and iodoacetate. Platelets from patients with thrombasthenia show a defective response to latex activated with either normal PPP or fibrinogen. Particles of inulin which activate C in the same way as Z, but which absorb much less fibrinogen, cause no response of either normal PRP or washed platelets. Thus normal platelets, but not those from thrombasthenics, appears to possess a receptor for fibrinogen which is important in their interaction with particulate material.

ALTERATIONS IN THE TRANS-MEMBRANE POTENTIAL OF HUMAN BLOOD PLATELETS IN RESPONSE TO AGGREGATING STIMULI. W. C. Horne, N. E. Larsen, and E. R. Simons. Boston University School of Medicine, Boston, Massachusetts, U.S.A.

It has been suggested that the mechanism of response of platelets to aggregating stimuli is analogous to the stimulus-response coupling mechanisms of muscle and secretory cells. Because the coupling mechanisms of both types of systems involve changes in the trans-membrane potential of the stimulated cell, we have examined the membrane potential of human platelets to determine if the potential changes in response to aggregating agents. The membrane potential of gel-filtered platelets was monitored using a fluorescent cyanine dye. Aggregation was prevented to minimize artifacts due to changes in light scattering. The effects of ADP, thrombin, and collagen on the potential were examined. In response to ADP, the membrane rapidly became more negatively polarized. The response was concentration dependent, the minimum response occurring with $3x10^{-5}$M ADP. The response to thrombin was more complex. Low concentrations (0.01 U/ml) produced a change similar to that caused by ADP. Higher concentration (1-10 U/ml) led to a rapid decrease in the polarization of the membrane. When pre-formed collagen fibrils were added, in the presence of creatine phosphokinase to destroy released ADP, no change in the potential was observed.

Thus the platelet membrane potential changes in response to aggregating stimuli, supporting the hypothesis that stimulus of platelet aggregation is analogous to other stimulus-response coupling mechanisms. The change in potential is not a necessary step in the coupling mechanism. The different responses to specific agents indicate that the agents may trigger aggregation through different mechanisms.

STUDIES ON THE INTERACTION BETWEEN (^{14}C)-EPINEPHRINE AND ISOLATED MEMBRANES FROM HUMAN PLATELETS H.Vainer-Institut de Recherches sur les Maladies du Sang, Hôpital Saint-Louis,75010 Paris,France

Previous studies have reported on the main characteristics of epinephrine's capacity to stimulate resting platelets. This study was designed to further investigate this problem using (^{14}C)-epinephrine (*E) and isolated membranes from human platelets. Membranes were isolated following the method of Barber & Jamieson (1970): a 6 and 2 fold enrichment in p (nitrophenyl) PDEase and ATPase and negligible levels of cytoplasmic marker enzyme activities have been measured. Incubations contained membranes and *E (33 mCi/mM) in Tyrode's solution at pH 7.4; membrane bound *E was quantitated by Millipore filtration. Binding was a rapid, reversible and saturable process: It reached equilibrium by 30 min. at 37°C; half-maximum displacement of *E occurred at $5x10^5M^{-1}$ "cold" E; about 400 pmoles of E were bound at saturation /mg of membrane protein/hour with a K_{aff}=$4.5-5.2x10^7M^{-1}$. Acid dissociated E from the labelled membranes showed that E structural alteration or degradation does not occur during binding. Impaired binding follows membrane exposure to impermeant -SH groups reagents or to trypsin, chymotrypsin, pronase and beta galactosidase. A specific binding appeared from its inhibition by alpha adrenoceptor agents, whereas beta active compounds had a much lower effect at higher concentrations; E metabolites did not inhibit binding. Mersalyl, a specific inhibitor of Mg^{2+}ATPase of the myosin-like platelet protein, Thrombosthenin M, strongly inhibited binding. It thus appears that E interaction with isolated platelet membranes has properties to be expected of binding to alpha adrenoceptors and involves thrombosthenin; binding occurred at E concentrations consistent with those reported to stimulate highly responsive platelets to E, suggesting that changes in stereochemical configuration of isolated membranes may cause exposure of new reactive sites for E.