FURTHER STUDIES ON THE GLYCOPROTEIN COMPOSITION OF NORMAL HUMAN, BERNARD-SOULIER AND THROMBASTHENIC PLATELETS. A.T. Nurden and J.P. Caen, Hopital Lariboisiere, Paris, France.

The glycoprotein composition of normal human platelets has been more precisely defined using SDS-polyacrylamide gel electrophoresis and comparing SDS-phosphate, SDS-tris and SDS-tris-glycine buffer systems. At least 10 glycoproteins may be identified including a previously unlocated high M. Wt. (in excess of 500,000 daltons) membrane glycoconjugate, glycocalicin and the intracellular thrombin sensitive protein. A much diminished staining for carbohydrate of membrane GP Ia and Ib, and of glycocalicin, were the major characteristics observed for Bernard-Soulier (B-S) platelets which also gave a reduced agglutination with wheat germ lectin. These abnormalities were much greater than the changes observed using neuraminidase-treated control platelets. The B-S platelets aggregated rapidly in the presence of thrombin, an event difficult to reconcile with glycocalicin being the proposed receptor for thrombin on the platelet surface. Membrane GP IIb and also GP IIIa (depending on the conditions of membrane solubilization and the subsequent SDS-polyacrylamide gel electrophoresis) both appeared to be absent in thrombasthenic platelet membranes. These abnormalities were often accompanied by an increased staining for carbohydrate of the members of the GP I family. With both the control and the pathological platelets the rate of migration and the staining intensities of the glycoprotein bands depended on the gel buffer system and the presence or absence of reducing agent during the initial solubilization. The results have indicated structural relationships and possible interconversions between individual GP bands. They have also more closely defined the glycoprotein abnormalities in B-S and thrombasthenic platelets.

AN INVESTIGATION OF THROMBASTHENIC PLATELETS WITH SELECTED LECTINS. B.G. Firkin and L. Stott, Monash Uni., Alfred Hospital, Melbourne, Australia & Dept. Pharmacology, Cambridge Uni., U.K.

Recent studies have identified a glycoprotein abnormality in platelet membrane preparations from patients with thrombasthenia. Whole blood was collected into iso-osmolar buffered formalin (0.1%) - the platelet rich plasma separated and stored at 4°C for 24 hours to ensure fixation with abolition of the release reaction as a possible factor in lectin aggregation. The platelets were washed 3 times and resuspended in Tris saline buffer pH 7.4. The cells were reacted with the lectins E-PHA, Ricinus communis I (RC-I) and Con A at varying concentrations in a Born aggregometer. Normal formalinised platelets (NFP) were strongly agglutinated by E-PHA and RC-I at concentrations of 50 and 15 µg/ml respectively. Con A at concentrations of 125-250 µg/ml also gave agglutination of NFP, but only to 30-40% of that with the other two lectins. Formalinised platelets prepared from 4 thrombasthenics all reacted normally with E-PHA and RC-I but were agglutinated minimally by Con A. Con A was labelled with I¹²⁵ and binding studies undertaken. A marked reduction of Con A I¹²⁵ uptake was observed with thrombasthenics, such that at saturation the binding sites of the lectin were approximately 25% that of NFP. Since trypsin digestion of platelet membranes results in an overall reduction of platelet glycoprotein, NFP were treated with trypsin. NFP treated in this fashion showed enhanced agglutination with E-PHA and RC-I and no discernable difference with Con A agglutination and binding of Con A I¹²⁵ was only slightly reduced. Con A may identify an important structural site in the core of the glycoprotein involved in platelet aggregation.