Rabbit and rat platelets have been extensively investigated under in vitro or in vivo conditions to try to understand the mechanism of platelet aggregation in man. Here, surface-labelling techniques have been used to find out if the platelet surface has a similar composition in these animals and in man or not. Human, rabbit and rat platelets were isolated, washed and surface-labelled by techniques specific for protein or for sugars (sialic acid or penultimate galactose/N-acetylgalactosamine residues). Labelled platelets were solubilized in sodium dodecyl sulphate and separated under reducing conditions on 7.5% Laemmli polyacrylamide gels. Dried gels were exposed to film by fluorography or indirect autoradiography. Terminal Gal/GalNAc residues (no neuraminidase treatment) were strongly labelled with rat and rabbit platelets compared to human platelets which labelled very poorly. Terminal sialic acid labelling with rat and rabbit platelets showed a weak labelling of a glycoprotein (GP) with the same M.Wt. as GPIIb which is the most intensely labelled GP in man. However, two GP with different M.Wts. (one GP is the only intensely labelled at a M.Wt. similar to that of GPIIa in man. These GP had a different M.Wt. with terminal Gal/GalNAc labelling. With a single GP GPIa and IIa in man were strongly iodinated with rabbit platelets but with rat platelets only a single band at the position of GPIIb was strongly iodinated. These results strongly indicate that there are considerable differences in surface composition between rabbit, rat and human platelets.

MEMBRANE GLYCOPROTEIN DEFECTS IN BERNAUD-SOULIER SYNDROME PLATELETS. K.J. Clezardin, J.L. McGregor, E. James, M. Dechavanne and E.F. Lüscher. Theodor Kocher Institute, University of Berne, Switzerland.

It is well established that Bernard-Soulier syndrome (BSS) platelets are deficient in a major membrane glycoprotein (Ib). In order to investigate if there is a deficiency of glycoprotein (Ib) in this disorder and to see if the β-subunit of glycoprotein (Ib) is also diminished, platelets from 3 BSS patients and from healthy donors were isolated, washed and surface labelled by lactoperoxidase-catalysed iodination, periodate/NaB₃H₄ or neuraminidase/galactose oxidase/NaB₃H₄. Labelled platelets were solubilized in sodium dodecyl sulphate and separated by 2-dimensional gel electrophoresis (isoelectric focusing, discontinuous polyacrylamide gel electrophoresis). Glycoprotein Ibβ was virtually absent in 2 patients and strongly decreased in the third patient. The β-subunit was also absent in the 2 patients and present at about 40% of normal in the third. Glycoprotein IIbβ was present normally in all patients. In addition, a further low molecular weight glycoprotein with a M.Wt. of 70,000 and a PI of 6.8-7.5 was absent or present at levels paralleling glycoprotein Iβ. The thrombin cleavable glycoprotein (GP IV or V) appeared greatly diminished with BSS platelets labelled by carbohydrate specific methods though no difference could be seen for the iodination of the band. These findings were confirmed in a fourth BSS patient using one dimensional gel electrophoresis.

The defects in BSS platelets are thus more complex than previously thought.

LECITHIN BINDING DEMONSTRATES DIFFERENT GLYCOPROTEIN ABNORMALITIES IN THROMBASTHENIC AND BERNAUD-SOULIER PLATELETS. Philip A. Judson and David J. Anstee. Department of Immunology, South Western Regional Blood Transfusion Centre, Bristol, U.K.

We have previously shown that the lectins from Maclura pomifera (Ma) and Arachis hypogaea (Ah) (peanut) bind selectively to GPIIb(3 and GPIIa(3. lectin binds primarily to GPIIb with no indication that GPIIa is absent or from the membranes of individuals with Bernard-Soulier Syndrome (B-S.S.) or GPIIa(3 and GPIIb(3 are deficient or absent from Thrombasthenic (G.T.) platelets. We have investigated the binding of radiolabelled lectins to SS6 polyacrylamide gels of whole platelets from patients with B-S.S. and G.T. The object of this study was to define further the nature of the glycoprotein abnormalities in these platelets.

The results clearly demonstrated a marked reduction in the binding of Ma and Ah lectins to the GPII region of B-S platelets when compared to that of normal platelets. No new lectin binding components were observed. The binding of Con A lectins to B-S platelets did not appear to be significantly different from that to normal platelets. In contrast Ma and Ah binding to G.T. platelets appeared normal whereas the binding of Con A to GPII-III was markedly reduced. Two of 3 G.T. binding bands were apparent in the GPI-II region of the G.T. platelets. It is not clear whether either of these represent residual GPII-III or if they are minor components masked by GPII-III in normal platelets. The carbohydrate content of GPII-III in normal and G.T. platelets appears to be entirely of O-glycosidically linked oligosaccharides. Ma and Ah lectins bind selectively to galactosyl and N-acetylgalactosaminyl residues in this type of oligosaccharide which suggests that B-S platelets have a gross deficiency of O-glycosidically linked oligosaccharides. Con A binds selectively to branched N-glycosidically linked oligosaccharides with a mannose-rich core. We conclude that the oligosaccharide present on GPII-III are grossly deficient in G.T. platelets.

FURTHER CHARACTERIZATION OF PLATELET MEMBRANE GLYCOPROTEINS. P. Clezardin, E. McGregor, K.J. Clezardin, M. Dechavanne and E.F. Lüscher. INSERM Unité 69. Fac. de Med., Alexis Carrel, Lyon, France and Theodor Kocher Institute, University of Berne, Switzerland.

The binding of ¹²⁵I-labelled lectins to major and minor platelet glycoproteins (GP) and their subunits has been investigated on platelets when compared to normal. Platelets were isolated, washed, solubilized in sodium dodecyl sulphate (SDS) under non-reducing conditions and separated on 5, 7.5 and 10% non-reduced/ reduced 2-D polyacrylamide gels. The gels were incubated with ¹²⁵I-labelled lectins: Lonicera japonica lectin (LCL), concanavalin A (ConA) wheat germ agglutinin (WGA) or Ricinus communis agglutinin (RCA-120), then washed extensively, dried and exposed to X-ray film by indirect autoradiography. WGA and RCA bound predominantly to GPIIb but also to two minor bands above and below it which were affected by neuraminidase treatment. One of them bound two ¹²⁵I-lectins (LCL and ConA) while GPIII did not. Additional GP bands were detected by lectin binding and by surface labelling beneath GPIIb and GPIII. With platelets labelled by the neuraminidase/galactose oxidase/NaB₃H₄ method a GP was detected between GPIa and GPIIa which disappeared in non-reduced/reduced 2-D polyacrylamide gels. The gel bands were incubated with ¹²⁵I-labelled lectins: Lonicera japonica lectin (LCL) and concanavalin A (ConA). GPIIb and GPIII were labelled equally strongly by surface labelling techniques. GPIb was apparently not labelled. Further GPI subunits were detected one below Iβ and IIb and another which originated in the GPVII region. These techniques demonstrate that the platelet surface protein is even more complex than previously thought.