K. Breederveld, J. Giddings**, J. W. ten Cate* and A. L. Bloom*** (* Division of Haemostasis, Dept. Haematology, Wilhelmina Gasthuis, Amsterdam, The Netherlands. ** Dept. Haematology, University Hospital of Wales, Cardiff, Great Britain): **The Interaction of Factor V with Human Blood Platelets. (501)**

The coagulant activity of gel filtered human platelets in the factor V assay is low. Lysis of these platelets by repeated freezing and thawing exposes a high coagulant activity, which is due to liberation of the factor V molecule from the damaged cells. Freezing and thawing of gel filtered platelets of a severe factor V deficient patient did not expose any coagulant activity.

Supernatants of centrifugated gel filtered platelet lysates contained specific factor V-antibody neutralizing capacity.

Fluorescence of normal platelets with FITC conjugated with sheep anti-rabbit immunoglobulin was inhibited by previous incubation of rabbit anti factor V-antibody with lysates of gel filtered normal platelets.

It is postulated, based upon these facts that factor V is localized intracellularly.

During collagen-induced aggregation of gel filtered normal platelets, endogenous serotonin is released within ten seconds, in contrast to factor V, which is slowly liberated, parallel with pf-3 activity.

J. N. George, P. C. Lewis and D. A. Sears (Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284, U.S.A.): **Membrane Glycoproteins of Human and Rabbit Platelets: Studies in Vitro and after Circulation in Vivo. (502)**

The initial events of hemostasis and thrombosis involve platelet contact interactions and may be mediated by surface glycoproteins. Human and rabbit platelets were labeled with ^{125}I-diazotized diiodosulfanilic acid (I), which reacts covalently with proteins, and proteins were separated by SDS-polyacrylamide gel electrophoresis. Only exposed membrane proteins were labeled because: 1) protein specific activity of membranes was 4–7 times that of whole platelets, 2) different proteins were labeled when I was reacted with isolated membranes, and 3) trypsin-hydrolysis of labeled intact platelets altered the radioactive peaks. Like Phillips (*Biochem. 11*, 4582, 72) and Nachman et al. (*JBC 248*, 2928, 73) we found that lactoperoxidase iodinated the 93,000 dalton glycoprotein (GP) of human platelets. In contrast, I labeled both the 93,000 and 118,000 dalton membrane GP of human platelets, and all 3 membrane GP of rabbit platelets.

Rabbit platelets labeled simultaneously with I and ^{51}Cr had identical density and therefore age distribution of the 2 labels. After infusion into rabbits, initial recovery of I was 23% of the Cr recovery. After 3 hrs, I disappearance was exponential and more rapid (T/2 = 17 hrs) than the linear Cr disappearance (T/2 = 30 hrs, $p < .01$). This was due to *in vivo* removal of I from circulating platelets since I did not elute more rapidly from platelets harvested after 3 hrs circulation and incubated in plasma at 37° (T/2 of I elution = 43 hrs, Cr = 33 hrs). Platelets harvested after 14–20 hrs circulation had the same distribution of I on the membrane GP as before circulation. We postulate that this symmetrical label loss indicates uniform loss of membrane GP, suggesting that platelets lose pieces of their plasma membrane during circulation. This could occur during contact interaction in the process of hemostasis.

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Adenosine is taken up tino intact washed human blood platelets by two independent carrier-mediated processes. The 'low Km system' has a Km of 9 uM, a V of 792 pMol/min/10^9, is energy dependent and has a Q_{10} of 1.71. This system is competitively inhibited by papaverine and dipyridamol. The high Km system has a Km of 9.4 mM, as V of 106 nMol/min/10^9 is also energy dependent and has a Q_{10} of 1.31. This system is competitively inhibited by adenine, that has a very high affinity (K_i = 5,8 uM).