MEMBRANE MICROENVIRONMENTAL CHANGES DURING PLATELET AGGREGATION: A STUDY WITH FLUORESCENT PROBES. I. Nathan, A. Dvilansky, G. Fleisher and A.H. Parola. The Soroka Medical Center and the Ben Gurion University of the Negev, Beersheva, Israel.

Studies were carried out with fluorescent probes which covalently bound with varied selectivity to plasma membrane proteins. Comparison with non-covalently bound fluorescent probes, Aniline naphthalene sulfonic acid and 1,6-diphenyl hexatriene, should reveal the role of various membranal constituents in the aggregation process. Combined phase contrast and fluorescent microscopy studies revealed essentially no alterations of platelet morphology, caused by the probes. Washed or sepharose 2-B separated labeled platelets exhibited normal aggregation curves. Fluorescent emission intensity decreased 3 to 4 fold during aggregation. The observed decay of fluorescence was not merely a result of the gross macroenvironmental changes in turbidity, as evident by the different initial rates of the processes. Rotational dynamics of the labeled plasma protein were followed by the fluorescence polarization method. An increase in fluorescence polarization values during initial aggregation stages was observed. These results may indicate the motion of the labeled proteins from the internal hydrophobic membrane region to the exposed hydrophilic environment resulting in the reduction of the rotational freedom of those labeled proteins.

BINDING OF DIPYRIDAMOLE TO HUMAN PLATELETS AND TO α_1-ACID GLYCOPROTEIN AND ITS SIGNIFICANCE FOR THE INHIBITION OF ADENOSINE UPTAKE. K. Subbarao, B. Rucinski, A. Summers and S. Niewiarowski. Temple University School of Medicine, Philadelphia, Pennsylvania, U.S.A.

The interactions of dipyridamole with α_1-acid glycoprotein of plasma and with human platelets are related to inhibition of adenosine uptake by platelets. One mole of dipyridamole binds to one mole of α_1-acid glycoprotein with a dissociation constant (K_d) of 1.3 µM. It was found that platelets contain both high and low affinity binding sites for the drug. The binding of dipyridamole to the high affinity sites follows a Michaelis Menten binding pattern with a K_d of 0.04 µM. Approximately $2x10^4$ dipyridamole molecules are bound at the high affinity sites of each platelet. The lower affinity sites bind the drug with a K_d of 4 µM. In the presence of α_1-acid glycoprotein the binding of dipyridamole to platelets is inhibited. Correspondingly, the dipyridamole inhibition of adenosine uptake by platelets is reduced 1000-fold by α_1-acid glycoprotein. Binding of dipyridamole to human platelets is essential for its inhibition of adenosine uptake by platelets. Dipyridamole reduced the [^{14}C]-ATP to [^{14}C]-ADP ratio in the platelets. Purified α_1-acid glycoprotein reversed these effects of dipyridamole on adenosine metabolism of platelets in a concentration dependent manner. A correlation was observed between the level of circulating dipyridamole in plasma and the inhibition of [^{14}C]-adenosine uptake by platelets of PRP samples of 12 human volunteers given different amounts of dipyridamole. The *in vitro* and *ex vivo* effects of dipyridamole on the [^{14}C]-adenosine uptake by platelets were found to be identical. Our data suggest the presence of dipyridamole binding sites in platelets that regulate adenosine transport across the cell surface.

PLASMA MEMBRANE VESICLES FROM MOUSE 15091A TUMOR CELLS: AGENT OF PLATELET AGGREGATING ACTIVITY. G.J. Gasic, J.L. Catafalmo, G.P. Gasic, S.J. Shattil, and G.J. Stewart. University of Pennsylvania School of Medicine and Temple University, Philadelphia, Pennsylvania, U.S.A.

We reported that cells from most tumors display platelet aggregating activity (PAA) in heparinized plasma and that this activity contributes to metastasis. Recently, we demonstrated that PAA can be used as a marker of cell transformation in virally infected rat cells. The material responsible for PAA is shed into culture medium. Characterization revealed a material which is particulate and sedimentable at 50,000x g for 60 min.; it contains proteins and lipids with a free cholesterol to phospholipid ratio of 0.556. Delipidation as well as complete solubilization abolished PAA. SDS-ME PAGE, 7.5% slab gels, revealed 20 bands.

EM studies of 50,000x g pellets shed by 15091A cells indicated they contained numerous vesicles, some solid bodies, numerous free or vesicle associated small particles, and some amorphous material. Discontinuous sucrose density gradient centrifugation of the 50,000x g pellet yielded at the 1.07-1.17 g/cm^3 interface a predominantly vesicular fraction which was the most active interfacial material. The vesicles, visible with phase contrast microscopy, resemble those produced by artificial plasma membrane vesiculation in various cell systems, including normal cells. Since PAA is only shown by transformed cells, vesicles from these must be different or much more numerous. Spontaneous vesiculation by tumor cells may be potentially important in understanding cell transformation and tumor metastases.