THE PARASINUSOIDAL LOCATION OF MEGAKARYOCYTES IN MARROW: A DETERMINANT OF PLATELET RELEASE AND A PHYSIOLOGIC VERSION OF VASCULAR INVASION AND METASTASIS. M.A. Lichtman and J.K. Chamberlain. Dept. of Medicine, University of Rochester School of Medicine, Rochester, New York, U.S.A.

Megakaryocytopoiesis occurs in the hematopoietic (extravascular) compartment of marrow. Thus, platelets must traverse the wall of the vascular sinuses of marrow to enter the circulation. We have examined mouse and rat marrow, fixed by rapid immersion so as to maintain anatomical relationships as close to the natural state as possible. Quantitative transmission electron microscopy (TEM) of random transections of femurs established that megakaryocytes reside less than 1 μ m from a marrow sinus wall with a probability unlikely to be the result of chance ($P < .001$). An intimate relationship exists between the megakaryocyte periphery and the abluminal surface of the endothelial lining cell. At the time of platelet release megakaryocyte cytoplasm invaginates (invades) and penetrates the endothelial lining cell. The penetrating cytoplasm is detached and enters the marrow circulation. From their dimensions in comparison to circulating platelets, the released cytoplasm represents a packet of platelets that undergoes further fragmentation in the circulation. The parasinusoidal location of megakaryocytes and the process of sinus wall penetration and platelet delivery was observed both by TEM and scanning electron microscopy.

These studies provide quantitative support for a specific anatomical arrangement of megakaryocytes in marrow. Moreover, the process of platelet release appears to be a physiological form of metastasis with invasion of vascular walls and vascular spread of cells, that are in this case amitotic.

POSTER SYMPOSIUM XIV

Thrombosis: Antiplasmins.

HUMAN PLASMIN AND PROTEASE INHIBITORS: INTERACTION IN A WHOLE PLASMA SYSTEM. R.F. Highsmith, C.J. Burnett and C.J. Weirich. University of Cincinnati College of Medicine, Cincinnati, Ohio USA

As a central event in the human coagulation and fibrinolytic pathways, intravascular proteolysis is normally limited by the presence of numerous circulating protease inhibitors. Plasmin, the fibrinolytic enzyme, is inhibited *in vitro* by purified preparations of antithrombin, Cl-esterase inhibitor, α_1 -antitrypsin, α_2 -macroglobulin and inter- α -trypsin inhibitor. Using trace amounts of 125 I-plasminogen and conventional gel filtration techniques, the elution profile of the labeled zymogen was studied in whole plasma before and after activation with urokinase (UK) or streptokinase (SK). Following activation, the major 125 I-plasmin peak shifted to a higher molecular weight fraction of plasma than before activation. Lysine-sepharose affinity chromatography (LSAC), SDS-gel electrophoresis and immunodiffusion studies on the peak radioactive fractions revealed that a small percentage of the 125 I-plasmin formed was bound to α_2 -macroglobulin while a majority was complexed to a component in plasma (mol. wt. $\sim 60,000$) immunologically distinct from the known human antiplasmins. Similar results were obtained when UK or SK activated plasma was directly subjected to LSAC without prior fractionation. Minor differences were seen when the rate of activation was varied. However, in the presence of heparin, a significant amount of 125 I-plasmin was complexed to antithrombin. These results, obtained in a whole plasma system, lend support for the presence of a new antiplasmin which is believed to be the same inhibitor recently described by others. Also, these data suggest that the other antiplasmins may play a minor, yet important role in the regulation of plasmin activity under different physiological conditions.