To examine the effect of flow on megakaryocytes (megas) exposed to extracellular matrix (ECM), we subjected megas on ECM to laminar flow in a chamber similar to that used to study platelets circulated over aortic subendothelium. Megas, harvested from guinea pig bone marrow by centrifugal stimulation and velocity sedimentation, were allowed to adhere to cover slides coated with ECM, which were then placed in a perfusion chamber. Medium was circulated over the megas on this surface for up to 18 hr. The cells were examined during the experiments by phase contrast and afterwards by scanning electron microscopy. With time, many attached megas developed a single, prominent, elongated pseudopod resembling the flagelliform processes observed in situ protruding into marrow sinusoids and also observed in circulating megas. In other experiments megas were introduced into the flowing medium and exposed to new ECM-coated cover slips mounted in the chamber. At shear rates of 10-200 sec\(^{-1}\), megas from the flowing suspension started to adhere to the ECM within 1 to 2 minutes. The number of attached cells continued to increase for several hours. Adhesion of megas under flow to the ECM was specific, since there was no adherence to glass, to glutaraldehyde-fixed ECM coated cover slips or to endothelial cells cultured on ECM coated cover slips. With time many of the megas developed the same type of pseudopods which formed in megas attached to ECM prior to flow exposure. The responses of megas to flow over ECM illustrate two aspects of megas behavior: the acquisition, even before platelet shedding, of the adhesive capacity of circulating platelets, and the possible roles of ECM and flow as anchor and inducer of platelet shedding.

### vWF-MULTIMER PATTERNS IN PLATELETS, PLASMA AND MEGAKARYOCYTES FROM RAT VISUALIZED BY IMMUNO-STAINING OF WESTERN-BLOTTED AGAROSE GELS

An established rat megakaryoblast line differentiates when grown at low serum concentrations into megakaryocyte-like cells with apparent release of thromboctye-like cytoplasmic fragments. To study the nature of intracellular vWF, cells grown at high (20%) and low(2%) serum concentrations were harvested, centrifuged and supernatants were subjected to SDS-agarose flat bed electrophoresis followed by Western blotting as rat plasma and identified rat platelet SDS-extracts. The intracellular membranes were then blocked by gelatine and incubated with goat anti-human vWF serum. As secondary antibody a peroxidase-conjugated rabbit anti-goat serum was used with o-dianisidine as chromogen. The patterns obtained were: 1) goat anti-human vWF antiserum effectively bound to rat vWF(2) vWF-multimer patterns of rat platelets and rat plasma were similar, demonstrating a pattern indistinguishable from that of human plasma. 3)vWF-pattern of megakaryoblasts differed markedly from the above pattern, demonstrating only the presence of monomeric and proteo-meric species of vWF.

Cells grown at 2% serum concentrations contained markedly more vWF per cell than cells grown at 20%, but the same pattern was seen in both cases.

### CHANGES IN PROTEOGLYCAN AND SULFATED PROTEIN SYNTHESIS DURING MEGAKARYOCYTE MATURATION IN VIVO

We investigated changes in sulfated proteoglycan (PG) and sulfated protein synthesis during megakaryocyte (MK) maturation in vivo by characterizing the (35S) labeled molecules in MKs and platelets (PLTs) obtained from 3 hr to 5 days after injection of guinea pigs with (35S) sulfate. Radioactivity in macromolecules was maximal in MKs 3 hr and in PLTs 3 days after the injection to be collected and solubilized in 6M urea/50mM Tris/0.2% Triton X-100/0.1% NaCl, and Pgs and sulfoproteins were resolved on SDS-PAGE by fluorography. The size of PG-1 molecules was a function of glycosaminoglycan (GAG) chain length. The appearance of the different size PG-1 molecules in PLTs was accounted for by their appearance from MKs over the same time period. Thus the size of the PG-1 synthesized by MKs decreased with MK maturation. The (35S)-PG-2 appeared in PLTs only 2-3 days after (35S) injection, had Kav near 0.07 on CL-6B, and had GAGs of the same average size as those of PG-1. The hydrophobic character of PG-2 suggests that it might be the membrane PG. PG-1 and PG-2 were separated by SDS-PAGE and identified by fluorography. The core proteins of PG-1 and PG-2 were identified by chondroitinase digestion and identified by SDS-PAGE and fluorography. The GAGs of PG-1 and PG-2 were almost entirely chondroitin 6-sulfate. The average size of PG-1 was 200,000 and its GAGs about 45,000.

The sulfated proteins (20-25% of total cell 35S) eluted in the wash-through of the DEAE-Sepharose column with 0.23M NaCl. Their isoelectric points were 4.0-6.5. They eluted as a small peak near the Vd and a broad major peak from Vk 0.3-0.6 on CL-6B column which could be identified as a single band on SDS-PAGE by fluorography. Digestion with Pronase or papain released small (35S)-labeled fragments, and the (35S) appeared to be associated with oligosaccharides. The sulfoproteins appeared in PLTs primarily 2-4 days after (35S) injection, and different proteins were labeled at different time points.