vWF-MULTIMER PATTERNS IN PLATELETS, PLASMA AND MEGAKARYOCYTES
FROM RAT VISUALIZED BY IMMUNO-STAINING OF WESTERN-BLOTTED AGAROSE
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An established rat megakaryoblast line differentiates when grown at low serum concentrations into megakaryocyte-like cells with apparent release of thrombocyte-like cytoplasmic fragments. To study the nature of intracellular vWF, cells grown at high (20%) and low (2%) serum concentrations were harvested, centrifuged and to study platelets cultured over aortic subendothelium. Megas, harvested from guinea pig bone marrow by centrifugal elutriation and velocity sedimentation, were allowed to adhere to cover slips coated with ECM, which were then placed in a perfusion chamber. Medium was circulated over the megas on this surface for up to 18 h. 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, 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 megag 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.

CHANGES IN PROTEOGLYCAN AND SULFATED PROTEIN SYNTHESIS DURING
MEGAKARYOCYTE MATURATION IN VIVO. B.P. Schrick, C.J. Welsh and T. Jenkins-West. Thrombosis Research Center, Temple Univ. School of Medicine, Philadelphia, PA, USA.

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, solubilized in 8M urea/50mM Tris/0.2% Triton X-100/10M NaCl, and pHs and sulfoproteins were separated by DEAE-Sephacel chromatography. PGs (65% of cell 35S) were eluted as two fractions, one (PG-1, 87%) with 4M Gdn HCl and another (PG-2, 13%) with 4M Gdn HCl/2% TX-100. The Kav of PLT PG-1 on Sepharose CL-6B shifted gradually from 0.18 to 0.10 from 1-5 days after (35S) injection, and the smaller and larger PG-1 species 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 disappearance 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 3 days after (35S) injection, had Kav 0.07 on CL-6B, but 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 obtained 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 core proteins of PG-1 and PG-2 were obtained 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-Sephacel column and with 0.2M NaCl. Their isoelectric points were 4.0-6.5. They eluted as a small peak near the 0A and a major broad peak from Kav 0.3-0.6 on CL-6B, and could be identified at least 9 distinct bands on SDS-PAGE by fluorography. Digestion with NaOH/NaBthal, Pronase or papain released small (35S)-labeled fragments, and the (35S) appeared to be associated with oigosaccharides. The sulfoproteins appeared in PLTs primarily 2-4 days after (35S) injection, and different proteins were labeled at different time points.