FUNCTIONAL CHARACTERIZATION OF MEMBRANE SIALYLTRANSFERASE ACTIVITY OF HUMAN PLATELETS. K.K. Wu, C. Ku and C. Smith. Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, U.S.A.

To evaluate the role of membrane sialyltransferase in the initiation of platelet aggregation, we studied the stimulatory effect of epinephrine and adenosine diphosphate and the inhibitory effect of aspirin on the platelet surface sialyltransferase activity. The enzyme activity was assayed under optimal conditions as determined previously. The enzyme activity was intact washed human platelets, CMP-¹⁴C-sialic acid, desialated fetuin, Mn^{2+} and buffer to a final volume of 1 ml. The enzyme activity was enhanced to 172% of control by $1\mu M$, 152% by $5\mu M$ and 146% by $10\mu M$ epinephrine. Adenosine diphosphate enhanced the enzyme activity to a lesser extent: 103% at $1\mu M$ and 113% at $5\mu M$. In contrast, aspirin inhibited the enzyme activity to 46% of control when $10\mu g/ml$ of aspirin was used. Higher concentrations of aspirin failed to cause further inhibition. In the in-vivo experiment, 600 mg aspirin was given to normal subjects and the surface enzyme activity was determined 12 hours later. The enzyme activity reduced to 43% following aspirin administration. Furthermore, we studied the enzyme activity in a patient with "aspirin-like" release disorder. While the mean surface enzyme activity of 10 normal subjects was 1.56 ± 0.21 (S.D.) $pmole \cdot hr^{-1}$ per 10^8 platelets, the enzyme activity of the patient was only $0.91 pmole \cdot hr^{-1}$. The results strongly suggest that the membrane sialyltransferase plays an important part in the initiation of platelet release reaction.

Ca^{2+} REGULATION OF BOVINE THROMBOSTHENIN. L. Muszbek, J. Kuźnicki and W. Drabikowski. Department of Pathophysiology, University School of Medicine, Debrecen, Hungary, and Department of Biochemistry of Nervous System and Muscle, Nencki Institute of Experimental Biology, Warszawa, Poland.

In order to reveal the type of its Ca^{2+} -regulation bovine thrombosthenin - natural platelet actomyosin - was investigated by competitive actin and myosin binding assay and by urea gel electrophoresis. The Ca^{2+} -sensitivity of low ionic strength Mg-ATPase activity of platelet actomyosin was not influenced by the addition of excess skeletal muscle actin free of regulatory proteins. In contrast, the replacement of platelet myosin by skeletal muscle myosin resulted in a hybrid actomyosin insensitive to Ca^{2+} . If actomyosin was reconstructed from crude platelet actin and skeletal muscle myosin again no regulatory effect of Ca^{2+} could be observed. In the presence of EGTA a fast moving band with the mobility corresponding to muscle troponin C (TN-C) was detected by alkaline urea polyacrylamide gel electrophoresis. However, if Ca^{2+} was added this protein, unlike TN-C, neither changed its mobility nor formed a complex even if muscle troponin I was included into the system. The experimental results indicate that our Ca^{2+} sensitive bovine thrombosthenin preparation does not contain TN-C like component and is not regulated by an actin -, but rather by a myosin-connected system.

BIOCHEMICAL PROPERTIES OF HUMAN PLATELET FACTOR 4. D.A. Walz, L.E. McCoy, and V.Y. Wu. Wayne State University School of Medicine, Detroit, Michigan, U.S.A.

Human platelet factor 4 (PF4) was isolated from outdated platelet concentrates to evaluate its biochemical properties. Platelets were washed in saline, lysed by freeze-thawing, and centrifuged to remove particulates from the lysate. PF4 was adsorbed on heparin immobilized on Sepharose. The yield of PF4, eluted in molar saline, was $9.3 \mu g/ml$ platelets. Polyacrylamide electrophoresis indicated a single-chain protein with an apparent molecular weight of 9,600. Amino acid composition indicated a 92 residue chain having 2 disulfide bridges and free of MET, PHE, and TRP. Electrophoresis of equal molar ratios of heparin and PF4 in the presence of 0.1% SDS resulted in the appearance of a slow migrating band having an apparent molecular weight of 20,000. The isolated PF4 was poorly soluble in the absence of heparin or at low salt concentrations. Elution profiles of gel and membrane filtration studies were indicative of molecular aggregation. PF4 appeared to be totally resistant to tryptic and chymotryptic hydrolysis but not to pepsin. However, PF4 bound to heparin was resistant to pepsin. Reduced-alkylated PF4 retained its ability to bind heparin but lost its resistance to tryptic or chymotryptic hydrolysis. The presence of heparin did not alter the peptide pattern following tryptic or pepsin hydrolysis of reduced-alkylated PF4 while the peptide pattern after chymotrypsin was modified. Peptides released after chymotryptic hydrolysis of reduced-alkylated PF4 had no apparent affinity for heparin unless heparin was present originally. These peptides after chymotryptic hydrolysis of heparin-bound-reduced-alkylated PF4 may serve as models for the design of small, synthetic heparin antagonists. (Supported by USPHS NIH grant #HL-20317-01 and the Charles DeVlieg Foundation.)