IMPORTANCE OF HUMAN PLATELET ECHINOCYTES FOR PROCOAGULANT ACTIVITY. E. Roth and H.M. Fraimovic. Department of Physiology, McGill University, Montreal, Canada.

Various agents can be used to transform platelets from the disc-form (discocytes) into spherical shapes from which many pseudopodia project (echinocytes). This change in shape normally precedes platelet aggregation and release. The relative importance of these changes on procoagulant activity was investigated. Recalcification times of platelet-rich plasma were used to monitor procoagulant activity, and echinocytes were maintained by the addition of either thrombin, serotonin, adenosine diphosphate (ADP) or water. The amounts of these agents added were insufficient to cause aggregation and release, and had no effect on the recalcification times of platelet-free plasma. Platelet shape and extent of aggregation were assessed from the stirring dependent changes in light transmission in an aggregometer, and by direct observation under a phase contrast microscope. When more than 60% of the platelets were echinocytes, recalcification times were shortened by at least 50%. Aggregates preformed with serotonin and ADP did not further shorten the recalcification times. The importance of the echinocyte for procoagulant activity is further supported by the observations that: 1) aggregates of discocytes formed with adrenaline caused only 20% shortening of recalcification times, 2) both discocytes (for discocyte preparations) and their aggregates appeared only during the last 10% of the total recalcification time, and 3) inhibition of the ADP-induced discocyte-echinocyte transformation by ADP prolonged recalcification times by 300%. These results suggest that platelet discocyte-echinocyte transformation is an important rate limiting step for platelet procoagulant activity in blood clotting.


The authors have determined the SH groups (P-SH) of the platelet membrane using four different methods (spectroscopic dosage, radioactive dosage, absorption and liquid phase electrophoresis). Molecules used: a) Molecules with disulfure bound. P-S-S-P. The global scheme of reaction of these molecules with SH groups is: P-SH + P-S-S-P -> P-S-S-P + 2 S0. The molecules used here were: DMN (2-thion-5' bis nitro 5 benzene acid), PDN (2-thion-5' pyridine) and CPMB (2-thion-6-6 diminosuccinic acid).

b) Chemical compounds which introduce an acid group instead of the SH group.

PCMB (para-chloromercury benzene sulphonamide) and PDMS (para-chloromercury benzene sulfonate). The mercury thus fixed is assayed by liquid scintillation with PCMB-14C and by absorption with PCMB.

Results: 1. 4,6 g. 3,1.10^-4 groups determined by electrophoresis (PCMB and PDMS).

- 6.1.10^-4 and 2.1.10^-4 groups assayed by chemical methods with DMNB, CPMB and EPN.

- 2.1.10^-3 groups determined with PDMS-14C.

These differences are discussed in relation to the accessibility of SH groups on and in membrane. This work was supported by BNFR (grant 16.34.146.00.A80.75.01)


An inter-laboratory estimation of F VIII potency has shown that the reagent was the main source of variation. The complicated nature of the F VIII reagent dictates that the "plateau of the optimal activation" to be determined experimentally. Thus it is essential to design a direct method to overcome problems due to the reagent variability and stability. 22222 is a highly sensitive substrate for F X, that has been successfully used instead of plasma substrate for measuring F X generation. The rate of cleavage was found to be strongly influenced by small changes in the volume of serum and phospholipid. This allows the possibility of using the reagent to obtain a specific rate of reaction. The optimal F X activation also depends upon the form in which the functionary active reagents (Ca2+, phospholipid, serum dilution) are added. Some commercially combined reagents gave very poor rate of cleavage and lack of slope for various dilutions of reference plasma. This makes the results invalid. The colourimetric test using 22222 is quick and sensitive. The results are reproducible. Thus the method fulfils the requirements for an acceptable test for monitoring and standardising the composition of factor VIII reagents.