IMPORTANCE OF HUMAN PLATELET ECHINOCYTES FOR PROCOAGULANT ACTIVITY. E. Toth and M.M. Frojmovic. 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 shape change normally precedes platelet aggregation and release. The relative importance of these changes for 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 echinocytes (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 AMP 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.

DETERMINATION OF SH-GROUPS OF THE PLATELET MEMBRANE. J.F. Stoltz and A. Nicolas.

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The authors have determined the SH groups (P-SH) of the platelet membrane using four different methods (spectrometric dosage, radioisotopic count, atomic absorption and liquid phase electrophoresis).

Molecules used: a) Molecules with disulfure bound (ϕ -S-S- ϕ). The global scheme of reaction of these molecules with SH groups is: $2P-SH + \phi-S-S-\phi \rightarrow P-S-S-P + 2\phi-S^{\cdot} + 2H^+$. The molecules used have been: DTNB (dithio-5-5' bis nitro 2 benzoic acid), PDS (dithio-2-2' pyridine) and CPDS (dithio-6-6' dinicotinic acid).

b) Mercurial compounds which introduce an acid group instead of the SH group PCMB (para-chloromercuribenzoic acid) and PCMBs (para-chloromercuribenzene sulfonic). The mercury thus fixed is assayed by liquid scintillation with PCMB- ^{14}C and by atomic absorption with PCMBs.

Results: - 2,6 to 3,1.10⁵ groups determined by electrophoresis (CPDS and PCMBs).
- 6,310⁷, 10.10⁷ and 26.10⁷ groups assayed by chemical methods with DTNB, CPDS and PDS.
- 2,310⁷ groups determined with PCMB- ^{14}C
- 1,1510⁷ groups calculated from atomic absorption

These differences are discussed in relation to the accessibility of SH groups on and in membrane.

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A COLOURIMETRIC TECHNIQUE FOR MONITORING VARIATIONS IN THE COMPOSITION OF F VIII REAGENTS.

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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 the "plateau of the optimal activation" to be determined experimentally. Thus it is essential to design a direct method to surmount problems due to the reagent variability and stability. S2222 is a highly sensitive substrate for F X_a that has been successfully used instead of plasma substrate for measuring F X_a 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 "tuning" the reactants to obtain a specific rate of reaction. The optimal F X_a activation also depend upon the form in which the functionary active reactants (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 S2222 is quick and sensitive. The results are reproducible. Thus the method fulfils the requirements for an acceptable test for monitoring and standardizing the composition of factor VIII reagents.