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PREDICTION OF STABILITY OF THROMBOPLASTIN. K.J.Stevenson. National (UK) Reference Laboratory for Anticoagulant Reagents and Control, Withington Hospital, Manchester, UK.

A new, rapid degradation method for predicting the stability of lyophilised extracts of tissue thromboplastin by accelerated oxygenation is described. This requires less time to complete and is more relevant to tissue thromboplastin age degradation than heat-accelerated methods previously advocated. Lipid oxidation is an important contributor to age-related change of thromboplastins. Although high temperatures employed in heat degradation may increase oxidative processes in thromboplastin lipids, the protein component essential for procoagulant activity is also denatured.

The practical application of the rapid oxygenation method as a technique for accelerated degradation of tissue thromboplastin reference preparations is described.

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THE EFFECT OF DIFFERENT ACTIVATORS AND THROMBOPLASTINS ON A CHROMOGENIC APTT. J.T. Brandt, D.A. Triplett, and J. Schaeffer. Department of Pathology, Ball Memorial Hospital, Muncie, Indiana, U.S.A.

Four different activator/thromboplastin reagents (silica/rabbit brain cephalin, kaolin/ simian brain cephalin, ellagic acid/rabbit brain cephalin, and ellagic acid/bovine brain cephalin) were evaluated for use in a chromogenic activated partial thromboplastin time (APTT). Parallel determinations using the same reagents in a clotting APTT were made. Compared to the clotting APTT, the chromogenic APTT using ellagic acid activation showed much greater sensitivity to both in vivo and in vitro heparin. Silica activation in the chromogenic assay showed decreased sensitivity to in vivo and in vitro heparin compared to the clotting assay. The kaolin/simian brain cephalin reagent was relatively insensitive in both clotting and chromogenic assays. A significant difference between in vivo and in vitro heparin was noted with all of the reagents in both clotting and chromogenic assays.

The chromogenic assays were more sensitive to the effect of vitamin K deficiency and coumadin administration than the corresponding clotting assays. Relative to the effect on the clotting APTT, the chromogenic assays were more sensitive to coumadin and vitamin K deficiency than to in vivo heparin.

The results indicate that the chromogenic APTT is not equivalent to the clotting APTT. Distinct differences in sensitivity to heparin and coumadin exist between the two assay systems. There is a marked difference in response to different reagent systems in the chromogenic assay. These various effects need to be considered when designing chromogenic assays.

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THE EFFECTS OF AN ANTICOAGULANT PHOSPHOLIPASE A_2 ON THE PROCOAGULANT ACTIVITY OF PHOSPHOLIPIDS AND PLATELETS. A.C.Cox, Departments of Biochemistry and Experimental Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Okla. USA.

Although phospholipids readily substitute for platelets in many in vitro blood coagulation tests, their participation in normal platelet procoagulant activity is uncertain. Phospholipase A_2 (PLA_2) from Naja nigricollis nigricollis venom, a known anticoagulant, blocked the enhancement of the rate of prothrombin conversion to thrombin by both phospholipids and platelets. The rate of inhibition of the phospholipid procoagulant activity by PLA_2 was reduced by indomethacin, an inhibitor of PLA_2. At concentrations of PLA_2 which inhibited phospholipid procoagulant activity, conversion of purified prothrombin by factors Xa and Va without phospholipid was unaffected.

Unactivated, washed platelets combined with coagulation factors II, Va and Xa initially produce thrombin at a slow rate before the platelets become activated but after this lag period the rate of thrombin generation increases. PLA_2 added at the same time as the coagulation factors increased the lag period and decreased the rate of thrombin generation during and after the lag. Preincubation of platelets with PLA_2 further decreased only the lag rate and this inhibition was partially blocked by 300 uM indomethacin. At a concentration of about one ng/ml, PLA_2 reduced the post-lag thrombin generation rate of 3×10^{-9} platelets/ml in half but had no effect on platelet aggregation induced by thrombin, ADP or collagen.

These results combined with the known specificity of PLA_2 support the theory that phospholipids are involved in platelet procoagulant activity. Furthermore, the ineffectiveness of preincubating PLA_2 with platelets on the post-lag procoagulant activity suggests that the phospholipids involved in this post-activation process become accessible during the lag period.

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PLATELET FACTOR V-LIKE COAGULANT ACTIVITY AND CATALYTIC PHOSPHOLIPID-LIKE SURFACE ACTIVITY IN CLOTTING AND CHROMOGENIC ASSAYS. F.A. Dombrose†, A.P. Bode†, H. Sandberg†, B.R. Lentz§, M.E. Jones§ and D.C. Crumpler†. Departments of Pathology and Biochemistry , Thrombosis and Hemostasis Center, Univ. of North Carolina, Chapel Hill, N.C. 27514, USA

The role of the platelet membrane as a catalytic surface in thrombin generation was studied, in vitro, using normal and F.V-deficient human plasmas. Frozen-and-thawed lysed platelet supernatant from normal or V-deficient donors was used as a reference for physiologic activation, in vitro. Coagulant activities were evaluated by both a clotting test (based on the KAPTT, in which the kaolin suspension was removed prior to recalcification) and by assessing thrombin formation (based on the maximum rate of hydrolysis of S-2238). The degree to which the clotting time was shortened or thrombin formation was enhanced was taken as overall catalytic surface activity. In both tests, log-log plots of the dependent variable as a function of the dilution of a given membrane preparation were linear over several orders magnitude ($p > .25$). The slopes of such plots were taken as overall catalytic surface effectiveness and were dependent on both the parametric level of F.V and the nature of the membrane. For the same membrane preparation, effectiveness in the clotting test was highly negatively correlated ($-r > 0.9$) with effectiveness in the S-2238 system (i.e., clotting time was inversely proportional to the maximum amount of thrombin generated). The change in effectiveness as a function of the parametric level of F.V was taken as the sensitivity of that surface to F.V. For a given control, both test systems appeared to be equally sensitive to the parametric level of F.V. On the basis of differences in catalytic effectiveness, it was possible to distinguish qualitatively between membrane associated F.V-like activity (platelet factor 1) and phospholipid-like catalytic surface activity (platelet factor 3). Discrete clotting time measurements, like single thrombin determinations, can not adequately describe the catalytic contribution of the platelet membrane in thrombin generation.