
Changes in the morphology of human platelets induced by Ristocetin (RIPA) have been analyzed at ultrastructural level by means of a tannic acid procedure. Clots were compared with aggregation and binding experiments. Modifications of these tests induced by apyrase, a monoclonal antibody (Mab) to GPIIb/IIIa and EDTA were also investigated.

Transmission electron microscopy reveals that ristocetin precipitates adhesive proteins on platelet membrane. An electron-dense deposit was noticeable within 20 seconds after ristocetin was added. When experiments were carried out in the aggregometer cuvette under stirring, groups of platelets become activated, change shape, and finally aggregate releasing part of their content. The morphology of aggregates did not differ from those formed in the presence of ADP.

Aggregation studies demonstrated that a Mab to GPIIb/IIIa modifies the extent and the rate of the aggregation curve when RIPA was performed in citrated platelet rich plasma (c-PRP). Apyrase modified the extent but not the slope of the curve. Neither the aggregation curve when RIPA was performed in citrated platelet poor plasma (c-PPP), nor the aggregation curve when RIPA was performed in PRP obtained in presence of EDTA. Binding experiments confirmed that I-vWF bound to GPIIb/IIIa complex, but the interaction of fibrinogen with this glycoprotein, and other mechanisms of the platelet function including exposure of GPIIb/IIIa complex, interaction of fibrinogen with this glycoprotein, and the contribution of the release reaction.

DISTRIBUTION OF INTRAMEMBRANOUS PARTICLES (IMP) ON PLATELETS IN CLOTS UNDER ISOMETRIC TENSION. James G. White, M.D. (1) and Isaac Cohen, Ph.D. (2). Departments of Laboratory Medicine and Pathology, Pediatric/University of Minnesota, Minneapolis 55455 (1) and Rehabilitation Institute, Northwestern University, Chicago, Illinois 60611 (2).

Development of isometric tension in platelet-rich clots is a manifestation of fibrin binding to the cells, as well as to platelet contact points. However, the nature of the platelet-fibrin association through which the force of contraction is transmitted remains unknown. A previous report suggested that clustering of the intramembranous particles (IMP) visible in replicas of freeze-fractured platelets in clots might represent sites of fibrin attachment and force transmission across the platelet membrane. In the present study platelet clots were prepared at various stages during development of isometric tension. Clots fixed in glutaraldehyde were combined with osmic acid and further processed for thin sections, or with glycerol, frozen in liquid nitrogen and freeze-fractioned in a Balzer's device. Thin sections revealed the longitudinal orientation of platelets and fibrin strands in the long axis of tension. Close associations between fibrin strands and extended portions of platelets were readily identified. However, no clearly discernable repetitive associations could be identified. Replicas of freeze fractured platelet clots under isometric tension revealed aspects of fibrin strands substructure and the platelet secretory pathway. However, no specific association between thrombin activated platelets in isometric clots, no evidence of IMP clustering could be identified. Experiments to identify thrombin in IMP in replicas of freeze fractured platelets does not appear related to fibrin binding to platelets and to transduction of contractile force from the interior of the platelet to fibrin strands and the development of tension in isometric clots.

FACTORS AFFECTING PLATELET VOLUME ANALYSIS. A. Wehmeyer and W. Schneiders, Dept of Internal Medicine, Univ. of Dusseldorf, D-4000 Dusseldorf, F.R.G.

Parameters of platelet volume have become widely available with the introduction of Automated Platelet Counters. However, various sample products used in vitro affect the activity of platelet release. Where it has been demonstrated that bovine platelets lack the OCS cluster when performed in citrated channels of a DTS. Ultrastructural procedures for cytochemical demonstration of platelet peroxidase and lucine 6 phosphate resulted in selective deposition of reaction product in channels of a DTS in bovine cells. Membrane complexes formed by interaction of the OCS and DTS in human platelets were absent. However, some elements of the OCS in bovine cells were located very close to the cell surface. Results of these studies demonstrate that bovine platelets possess a functional OCS involved in the formation of isometric tension in platelet clots.