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PLATELET VOLUME MEASUREMENTS: THE IMPORTANCE OF ANTICOAGULATION. C.B. Thompson, D.D. Diaz, P.G. Quinn, M. Lapins, S.B. Kurtz and C.R. Valeri. Naval Blood Research Laboratory, Boston University School of Medicine and University Hospital, Boston, Massachusetts

Recent advances in electrical cell sizing have made mean platelet volume (MPV) routinely available in most clinical laboratories. To study the importance of anticoagulation on platelet size stability, blood was collected in 7 different anticoagulants and stored at room temperature for up to 8 hours. Platelet counts and platelet sizing were performed using whole blood on a Coulter S^+ and using platelet-rich plasma on a Coulter H4 Channelyzer. The results suggest that both calcium chelation and acidification were required to inhibit platelet shape change and aggregation. Electrolyte composition, pH, and tonicity of the anticoagulant all influenced the stability of the MPV. As a result of these studies, an anticoagulant combining ACD and Na_2EDTA at a pH of 5.0 and an osmotic strength of 308 mOsm/l was used to study platelet volume and counts in whole blood on a Coulter S^+ used in the hematology laboratory of our hospital. Platelet counts with ACD-Na_2EDTA anticoagulant were no different from routine Na_2EDTA platelet counts and exhibited 2.9% error in reproducibility and a 4.4% variability over the 8 hours of storage. Mean platelet volumes were reproducible to within 3% and had less than 1% variability over the 8 hours of storage. Platelets anticoagulated with ACD-Na_2EDTA remained discoid in shape and could be shown to undergo a shape change on stimulation with ADP up to at least 8 hours after collection, when the pH was adjusted to 7.4 and the Ca^{++} concentration restored.

These data demonstrate that platelet count and platelet volumes remained stable in blood collected in ACD-Na_2EDTA anticoagulant for up to 8 hours at room temperature. In 52 volunteers studied, an inverse correlation ($r=.72$, $p<0.001$) was observed between platelet count and MPV, suggesting that the circulating platelet mass may be a more important indicator of platelet homeostasis than either the platelet count or the mean platelet volume alone.

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A PLATELET AGGREGOMETER WITH AUTOMATIC DATA PROCESSING. P. Blasberg, L.J. Wurzinger, K. Mussler, H. Myrenne and H. Schmid-Schönbein. Dept. of Physiology, RWTH Aachen, F.R.G.

A new aggregometer was designed to meet the following requirements: A) a uniform well defined shear rate throughout the whole PRP sample, B) two different shear rates - one favourable for platelet aggregation (PA) - one to subject already formed aggregates to high shear to test aggregate stability, C) a modular set up to extend the aggregometer easily from 1 to 4 channels, D) automatic processing and documentation of all aggregation data.

The aggregometer consists mainly of two parts:
1. The aggregation moduls: Each aggregation modul contains a Couette-viscometer (500 µl PRP required). The outer cylinder (a disposable plastic vial) is rotated to induce shear rates in the range of $40s^{-1}$ and $4000s^{-1}$. For measurement of PA each channel is equipped with a He-Ne laser which radiates a light beam through the sample. Transmitted (I_t) and scattered (I_s) light is measured by independent photodiodes and from the ratio I_s/I_t+I_s turbidity of the test sample is estimated. The moduls are thermostatised to 37°C. 1 to 4 moduls are supplied by one motor-gear box (1:100) unit.
2. The data processing unit: The data processing unit is controlled by an OEM-microcomputer, which allows an on line display and storage of the actual turbidity values during the whole experiment. At the end of each experiment the aggregation curve is calculated and may be plotted on a x,t-recorder. The area under the curve and the lag phase of PA is estimated. In addition the whole experimental run is controlled and documented by the printer equipped microcomputer using the interactive high level language BASIC.

The described aggregometer exhibits the following advantages: the use of a uniform low shear rate enables one to observe spontaneous PA and to reduce the concentrations of PA-inducing agents (e.g. $5x10^{-7}$m ADP) compared to conventional aggregometers whereas the stability of already formed aggregates can be tested with the high shear rate. Furthermore the evaluation of all data (PA, integral of PA, lag time) is done automatically.

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STIMULATION OF DNA SYNTHESIS IN MOUSE FIBROBLASTS BY PURIFIED PORCINE PLATELET SECRETED PROTEINS. A. Poggi, B. Rucinski, J.C. Holt and S. Niewiarowski. Thombosis Research Center, Temple University Health Sciences Center, Philadelphia, PA 19140.

Human platelets secrete proteins which stimulate multiplication of fibroblast and smooth muscle cells and appear to be involved in the development of atherosclerosis. We have studied the effect of two purified porcine platelet secreted proteins on DNA synthesis in mouse fibroblasts (3T3 cells). Material released from washed platelets stimulated by thrombin was fractionated with 0.04 M $ZnSO_4$, followed by chromatography on Sephacryl S-200 and heparin-agarose. A number of proteins were eluted from heparin-agarose with a gradient of increasing NaCl concentration including Heparin Binding Protein (HBP) and Platelet Factor 4 (PF_4), eluted at 0.7 M and 1.1 M NaCl respectively. Both proteins were homogenous in immunoelectrophoresis and in SDS polyacrylamide gel electrophoresis. Their apparent molecular weights were 6,000 and 14,000 respectively. They showed no proteolytic activity using fibrinogen and D-Phe-Pip-Arg-pNa (S2238) as substrates. At concentrations 500-5000 ng/ml, HBP stimulated ^{3}H-thymidine incorporation into subconfluent 3T3 cells made quiescent by serum deprivation. At the latter concentration the effect was similar to that induced by 3-5% fetal bovine serum. Addition of HBP (5000 ng/ml) also stimulated proliferation of 3T3 cells plated in 1% of fetal bovine serum. In both assays porcine platelet factor 4 was at least 10 times less active than HBP. Mitogenic activities of human platelet basic protein and of human platelet derived growth factor were potentiated by addition of fetal bovine or human serum. By contrast, no synergistic effect was found between these sera or porcine serum and HBP (500 ng/ml) when tested by ^{3}H-thymidine incorporation assay. A possible role of secreted porcine platelet proteins in the development of atherosclerosis in swine is under investigation.

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PLATELET FACTOR 4 (PF4) RELEASE IN VITRO FROM UNSTIMULATED HUMAN PLATELETS. D.S. Cohen, J.M. Strohschein, R.N. Saunders and D.I. Cargill. Section of Platelet Research, Sandoz, Inc., East Hanover, N.J., U.S.A.

Human venous blood was collected (9.5:0.5) in an anticoagulant (provided in the PF4 RIA kit, Abbott Labs.) consisting of 2.5% EDTA, 0.025% adenosine and 7.0% procaine HCl. Platelet rich plasma (PRP) was stirred (900 RPM) at 37°C in a Payton aggregometer for intervals of 3, 6, 9 and 12 minutes. Unincubated aliquots of PRP were taken as the 0 time controls.

A significant ($p < 0.02$) elevation in PF4 was observed with increasing intervals. Furthermore, from the data obtained, two populations of PF4 releasers were delineated: A high releasing (HR) group and a low releasing (LR) group. HR (n=10) displayed a baseline release of 10.3 ± 0.8 ng/ml and values of 36.1 ± 9.0 ($p < 0.05$ compared to baseline), 46.8 ± 8.2 ($p < 0.01$), 54.6 ± 7.9 ($p < 0.01$) and 87 ± 13.1 ($p < 0.01$) for the corresponding time periods. LR (n=7) exhibited significant but diminished release at each time period with a basal level of 10.1 ± 1.0 and 14.7 ± 1.4 ($p < 0.05$ compared to baseline), 15.3 ± 1.5 ($p < 0.05$), 19.7 ± 2.4 ($p < 0.05$) and 23.6 ± 2.0 ($p < 0.01$) respectively. Comparison of both populations revealed significant ($p < 0.01$) differences at the 6, 9 and 12 minute intervals. Total PF4 content from Triton lysed PRP displayed similar levels between groups: 360.0 ± 22.6 (HR) and 380.2 ± 15.2 (LR).

We conclude that an endogenous release of PF4 occurs in vitro in unstimulated human PRP even in a milieu designed to deter release. This should be a concern when PF4 values are to be assessed.