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IN VIVO MEASUREMENT OF PLATELET AGGREGATION. J. L. Ambrus, C.M. Ambrus, H. Gastpar, P. Spavento, S. Gordon, and F.J. Weber. Roswell Park Memorial Institute, Buffalo, NY 14263 and the State University of New York at Buffalo, Buffalo, NY USA.

In stumptailed monkeys (Macaca arctoides) arteriovenous anastomoses were established. Into these an apparatus was inserted in which blood flows through a 20 micron pore diameter screen and blood pressure is recorded before and after the screen. Injection of 0.1 to 0.5 µgm of ADP or 1 to 2 µgm of serotonine resulted in rapid platelet aggregation on the screen even in heparinized animals. This increased pre-screen pressure and decreased post-screen pressure as recorded with strain gauges. From these data a platelet aggregation index is calculated. Platelet aggregation was significantly decreased by a series of agents which inhibit phosphodiesterases and stimulate release of prostacyclin. This includes several pyrimidopyrimidine derivatives, methylxantine derivatives, and imidazoquinazolinones. These agents increased circulation time of intravenously injected labeled polyploid ascites tumor cells. They decreased pulmonary embolism and thrombocytopenia following intravenous injection of tumor cells and decreased development of pulmonary metastases. These methods appear to be suitable to study platelet aggregation inhibitors and their mechanisms of action in vivo.

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THE INFLUENCE OF ANTICOAGULANTS UPON THE RESULTS OF PLATELET FUNCTION TESTS: AGGREGATION (PA) AND RELEASE REACTION (RR). L.J. Wurzinger and P. Blasberg. Dept. of Physiology, RWTH Aachen, F.R.G.

Citrate (11 mM) is generally used as anticoagulant when platelet functions are tested. In contrast to heparin anticoagulation, citrate drastically lowers calcium levels creating a milieu, incompatible with life in an organism. However, the effects of heparin on platelet function are still a matter of debate, studies on this subject employing variable protocols of heparin administration and thus probably being responsible for the existing conflict of results. The present study attempts a systematic comparison of spontaneous as well as ADP and adrenaline induced PA and the RR under influence of either heparin or citrate used as anticoagulant. Spontaneous and ADP or adrenaline induced (each 5×10^{-7} M) PA were tested in a 2-channel rheoaggregometer simultaneously on platelet rich plasma (PRP) samples prepared from blood anticoagulated with 5 U heparin/ml or 11 mM citrate. ADP and adrenaline induced PA were tested 90 and 60 min after blood withdrawal respectively. Spontaneous PA was tested 30, 60, 90, 120 and 180 min after blood withdrawal.

RR was assessed by measuring β-thromboglobulin (β-TG) release from gel filtered platelets, incubated for 30 min with varying doses of heparin (1,5,50 U/ml) or citrate (5,11,22 mM). Strict isothermia of 37°C was kept during all steps of the experimental procedure.

We found that:

1. Adrenaline and ADP induced aggregation were significantly higher in heparinized than in citrated PRP.
2. Spontaneous PA in heparinized PRP too was higher than in citrated PRP during the interval looked upon.
3. Heparin added to gel filtered platelets enhanced, whereas citrate lowered β-TG release as compared to controls.

From our results we conclude that the differences in the behaviour of heparinized and citrated PRP are not only due to an effect of heparin itself, but also to the difference in calcium levels.

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RETENTION OF PLATELETS IN A PROTEIN A-SEPHAROSE COLUMN AS A MEASURE OF PLATELET ASSOCIATED IgG. C.N. Chesterman and D.J. McVeigh. Melbourne University Department of Medicine and Haematology Department St. Vincent's Hospital, Melbourne, Australia.

Employing the biological property of staphylococcal protein A to bind human IgG a test system has been devised to detect platelet associated IgG (PAIg) by quantitating platelet retention in an agarose column with immobilised protein A.

Platelets were washed free of plasma and resuspended in Tyrode's buffer with EDTA and 0.1% BSA. Platelet counts were performed on the suspension prior to and following passage through a miniature protein A column and the percentage of platelets retained calculated. An indirect test for serum platelet antibody utilised washed normal platelets following their incubation in patient serum.

Less than 30% normal platelets or normal platelets incubated in normal serum were retained in the affinity column. Similarly in four samples from patients with thrombocytopenia of nonimmune origin there was less than 15% retention in the column. Platelets from two of five patients with probable ITP (all treated with corticosteroids) showed 85% retention in the column. The remaining patients fell within the normal range. The sera from three patients with proven platelet antibody using other techniques produced 65-80% retention in an indirect test. Four other sera from possible ITP patients (with comparatively high platelet counts) showed less than 30% retention. Specificity for PAIg was suggested by inhibition of retention from 80% to less than 30% following pretreatment of the column with purified IgG or normal serum.

The optimum conditions for the technique are being studied and its specificity and sensitivity ascertained in patients with thrombocytopenia of various kinds. If these preliminary results are extended, such a simple technique may prove of diagnostic value.

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LOSS OF PLATELET MEMBRANE GLYCOPROTEINS OR TERMINAL SIALIC ACID DETECTED BY FITC-LECTINS. L. McGregor, J.L. McGregor, K.J. Clemetson, M. Dechavanne and E.F. Lüscher. INSERM Unité 63, Fac. de Med. Alexis Carrel, Lyon, France and Theodor Kocher Institute, University of Berne, Switzerland.

Pre-thrombic conditions in certain individuals resulting from enhanced platelet-vessel wall or platelet-platelet interactions are perhaps characterized by a reduction in certain membrane glycoproteins or loss of terminal sialic acid. In order to investigate if such changes are detectable, the binding of FITC-lectins to human platelets treated under in vitro conditions with certain proteases to mimic possible in vivo changes occuring on the platelet surface, has been examined. Human platelets were isolated, washed and either treated with neuraminidase (10 U) or plasmin (1 CU) before fixing with formaldehyde. Binding studies were performed by the method of Monsigny et al. using FITC labelled wheat germ agglutinin (WGA), Lens culinaris lectin (LCL), Ricinus communis agglutinin (RCA) and concanavalin A (ConA). The number of lectin-binding sites (n) and the dissociation constant (Kd) were obtained by Steck and Wallach reciprocal plots. After neuraminidase or plasmin treatment n was reduced but Kd remained approximately the same with WGA. FITC-RCA-60 gave a slight fluorescence with untreated and very strong fluorescence with neuraminidase treated platelets. Platelet glycoproteins separated by 2-dimensional gel electrophoresis were identified by binding of fluorescent lectins. Plasmin decreased the intensity of GP Ib and IIb and removed Ia completely. Neuraminidase decreased the labelling of Ib by WGA. These techniques show promise as methods of detecting pre-thrombotic conditions.