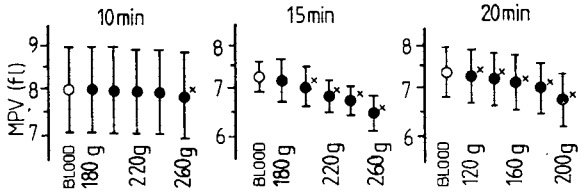
1750

PREPARATION OF A REPRESENTATIVE PLATELET POPULATION BY A SINGLE STEP SLOW CENTRIFUGATION. I. Denfors, H. Wadenvik and J. Kutti. Department of Medicine, Östra Hospital, University of Göteborg, Göteborg, Sweden.

Circulating platelets differ with respect to size, density and functional ability. In several experimental settings it is of vital importance that a representative platelet-rich plasma (PRP) is extracted from the anticoagulated blood. We therefore investigated the effect of centrifugation time and gravitational force on platelet yield and platelet volume distribution in PRP obtained by a single step slow centrifugation.

Methods. From each of 12 healthy male blood donors, 357 ml of venous blood were mixed with 63 ml citrate phosphate dextrose in a plastic bag. 20 ml aliquots of the anticoagulated blood were transferred into 30 ml screwcap polycarbonate tubes (Nalgene Labware). PRP was prepared by a single step slow centrifugation in a microprocessor-controlled bench centrifuge (Hettich Rotanta/RP). The gravitational force was calculated at the bottom of the tube. Centrifugations were performed at 180, 200, 220, 240, 260 g for 10 min, at 180, 200, 220, 240, 260 g for 15 min and at 120, 140, 160, 180, 200 g for 20 min. Three hours after blood collection, the platelet count, mean platelet volume (MPV) and platelet distribution width were determined in PRP and anticoagulated blood with an impedance cell counter (Coulter Counter Model S-Plus VI). Student's t-test was employed for comparison of mean values.

Results



Mean ± SD. x $p < 0.05$

Comments. Very precise centrifugation conditions can be provided by using a microprocessor-controlled centrifuge and a "fully representative" platelet population can be isolated with a single step slow centrifugation method.

1751

PLATELET AGGREGATION DOES NOT CONFORM TO SIMPLE PARTICLE COLLISION THEORY

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Platelet aggregation kinetics, according to the particle collision theory, generally assumed to apply, ought to conform to a second order type of rate law. But published data on the time-course of ADP-induced single platelet recruitment into aggregates were found not to do so and to lead to abnormal second order rate constants much larger than even their theoretical upper bounds. The data were, instead, found to fit a first order type of rate law rather well with rate constants in the range of 0.04 - 0.27 s^{-1}. These results were confirmed in our laboratory employing gelfiltered calf platelets. Thus a mechanism much more complex than hithertofore recognized, is operative. The following kinetic scheme was formulated on the basis of information gleaned from the literature.

$$P + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PA \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P^* \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} P^{**} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \text{Aggregates}$$

where P is the nonaggregable, discoid platelet, A the agonist, P* an aggregable platelet form with membranous protrusions, and P** another aggregable platelet form with pseudopods. Taking into account the relative magnitudes of the k's and assuming aggregation to be driven by hydrophobic interaction between complementary surfaces of P* and P** species, a rate equation was derived for aggregation. The kinetic scheme and the rate equation could account for the apparent first order rate law and other empirical observations in the literature.

1752

COMPUTERIZED PLATELET AGGREGOMETRY.

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One of the commonly used in vitro tests for assessing platelet function is a photodensitometric assay first established by Born. However the collection and analysis of aggregation data are tedious and time consuming. The single- and dual-channel aggregometers have limited utility for the analysis of large numbers of plasma samples in clinical laboratories or in studies on the mode of action of drugs.

We now report on a multichannel platelet aggregometer system consisting of an IBM XT personal computer and three microprocessor-controlled 4 channel aggregometers. The system collects, displays and analyzes 12 different aggregation curves simultaneously, and - like other computerized systems - (1) significantly increases the efficiency and ease in performing the experiment, analyzing and presenting the data; (2) provides systematic storage and rapid retrieval of the data; (3) saves an enormous amount of time; (4) because of multichannel capability eliminates the effects of time-related changes in PRP on the dose-response curves.

However our system has some advantages over the computerized aggregometer systems used so far: (1) since the aggregometers contain built in microprocessors they can be utilized to measure and analyse platelet aggregation without being coupled to a computer; (2) a special program helps to check the validity of the calculated parameters under visual control; (3) the individual points of the dose-response curves can be checked at any time during the experiment.

1753

RED BLOOD CELL LYSIS MAY INFLUENCE PLATELET AGGREGATION IN WHOLE BLOOD AGGREGOMETER. A.G. Herman and H. Bult, Univ. of Antwerp (UIA), Div. of Pharmacol., Wilrijk, Belgium.

The electronic whole blood aggregometer (WBA) has the advantage that it enables the study of platelet aggregation in whole blood shortly after blood collection. Using the WBA varying results have been obtained with respect to the anti-aggregating activity of dipyridamole. As dipyridamole is an efficient inhibitor of adenosine uptake, we tested whether the degree of red blood cell lysis (and thus availability of adenine nucleotides) affected its efficacy. Citrate (10.7 mM) blood was stored in sealed tubes and used between 20 and 100 min after venipuncture. One ml was placed in a Chronolog Model 540 WBA together with 10 µl 0.9 % NaCl, dipyridamole (final conc. 3, 10 or 30 µM) or its solvent (final conc. 0.03, 0.1 or 0.3 %). After reaching a stable baseline and WBA calibration, aggregation was induced by injection of 10 µM ADP dissolved in 10 µl 0.9 % NaCl (one channel) or 10 µl distilled water (other channel). Maximum impedance increase in 10 min was measured, red blood cells were removed by centrifugation, and from microhematocrit and absorbance at 416 nm the volume of lysed packed red blood cells was estimated. ADP caused aggregation (12.9 + 1.9 and 11.1 + 0.8, Ohm) and there was red blood cell lysis (2.8 + 0.5 and 0.8 + 0.2 µl red blood cells, ADP resp. in H_2O and 0.9 % NaCl, n = 6). Dipyridamole (30 µM) suppressed aggregation when compared with solvent, but only when ADP was given in H_2O (reduction resp. 4.4 + 1.4 and 1.9 + 1.6 ohm). Moreover, there was a negative correlation between the degree of haemolysis and the aggregation response at 10 as well as 30 µM dipyridamole. This reduced aggregation with increasing haemolysis was not observed in the presence of the corresponding solvent concentrations. The red blood cell lysis was proportional to the plasma ATP (luciferine-luciferase method) concentration as an index of adenine nucleotide leakage. In conclusion, a certain degree of haemolysis caused by stirring and injection with a microsyringe is inherent to the WBA, but use of hypotonic vehicles should be avoided. Release of red blood cell constituents may affect platelet aggregation as such, or interfere with the activity of adenosine uptake inhibitors and possibly other drugs. It may help to explain some of the variable results obtained with dipyridamole.