Anticoagulant effects on the measurement of erythrocyte filterability

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The data we presented at the 1982 Workshop indicated that erythrocyte deformability, as measured by positive-pressure filtration through 5 μm pore diameter polycarbonate (Nuclepore) membranes, was affected by the anticoagulant used during storage of the whole blood sample at room temperature (1). Subsequent analysis of the pressure–time curves of this system showed that the initial pressure (steady state slope of filtration curve extrapolated back to zero time; Pi) was the most sensitive of the curve parameters to these anticoagulant effects.

Twelve normal blood samples were therefore taken into K₂EDTA (1.5 mg/ml added blood; Seward Laboratory, UAC House, Blackfriars Road, London SE1 9UG) and stored for 6 hours at room temperature; there was no significant change in the mean Pi value with time (Fig. 1). When the same 12 bloods were taken into lithium heparin (15 IU/ml added blood; Sterilin Ltd., Teddington, Middlesex), there was a significantly lower (P<0.05) value for Pi (Fig. 1) at 1 h from venepuncture (heparin – mean 3.41, SEM 1.04; EDTA – mean 4.22, SEM 0.18). At 2 h and thereafter, the Pi values for heparinised blood had significantly increased so that there was no longer any significant difference from EDTA blood (Fig. 1). While this study (2, 3) showed an obvious difference between the two anticoagulants, it was not clear whether the difference reflected an anticoagulant effect on the filtered erythrocytes or on contaminating platelets or leucocytes.

We therefore studied the effect of storage of whole blood in EDTA and heparin on platelets and leucocytes. For EDTA samples, taken from 12 normal individuals, there was no significant change in mean platelet count over 6 h (Fig. 2) whereas 12 blood specimens taken into Vacutainer heparin (Becton Dickinson Vacutainer Systems, Rutherford, New Jersey, USA) showed thrombocytopenia (Fig. 2), which was a consequence of platelet microaggregate formation (2). It is known that heparin-induced platelet microaggregates can cause blockage of the 5 μm diameter pores of polycarbonate membranes (4).

Leucocytes were also found to be adversely affected by heparin in a time-
Fig. 1. Effect of storage of whole blood in EDTA (▲) and heparin (●) for up to 6 hours at room temperature on the initial pressure (Pi) of positive-pressure erythrocyte filtration.

dependent manner. Leucocytes stored in heparin at room temperature showed earlier morphological evidence of degeneration, such as cytoplasmic vacuolation, than did leucocytes taken into EDTA. Blood taken into Vacutainer heparin, but not EDTA, showed a progressive fall over 6 h in mean total leucocyte and absolute neutrophil counts, as determined by the Technicon Hemalog D system (Fig. 2). The neutrophil counting system of this instrument depends on myeloperoxidase cytochemical staining at 55°C and pH 3.2 which may have caused selective disruption of heparin-damaged neutrophils since visual leucocyte counts in a Neubauer chamber showed no significant leucopenia during 6 hours’ storage in either EDTA or heparin.

Finally, we studied the positive-pressure filtration of washed erythrocyte suspensions enriched with leucocytes taken from autologous buffy coat preparations. At 3 h from venepuncture there was no change in filterability for blood originally taken into EDTA, whereas leucocyte-enriched erythrocyte suspensions prepared from heparinised blood showed 32–72% loss of filterability after 3 h (2). Heparin thus adversely affected leucocyte filterability in a time-dependent way.

For the above reasons, it is necessary to remove all contaminating platelets and leucocytes from erythrocyte test suspensions if the effects of anticoagu-