1 Introduction
It is an attractive hypothesis that reduced red cell deformability in diabetics could contribute to the pathology of diabetic retinopathy. The small vessel diameters in the retina should be critical parts of the microcirculation, and allowing for the fact that the early diabetic could compensate initially by vasomotor control, when the vasodilator reserve is used up as in the long-term diabetic adverse effects would be expected (Schmid-Schönbein and Volger, 1976). To assess the cellular deformability of bulk blood the most usual technique involves micropore filtration, typical parameters being 5–20 cm H₂O driving pressure through a 5 μm pore diameter filter at 10–45 per cent haematocrit (Schmid-Schönbein and Volger, 1976; Gregersen et al., 1967; Barnes et al., 1977; Juhan et al., 1978).

This paper describes a series of tests to measure the deformability of a set of human blood samples in a filtration system using a low-pressure differential (2 cm H₂O), a volume flow detector sensitive to 20 μl volume changes and with sample preparation including white cell removal and haematocrit standardisation.

2 Materials and methods
2.1 Preparation of blood samples
Venous blood samples (10 ml) were taken into sodium heparin solution from patients and controls (5000 units ml⁻¹) during a morning session. At midday the samples were processed (within 2 h). As the effect of diabetes on specifically red cell flexibility was to be assessed white cells and other debris were removed before filtration. Also, as the reduction of filtration rate with increased haematocrit was well documented (Drummond et al., 1980; Kikuichi et al., 1979), typically a 10 per cent flow rate change for 5 per cent haematocrit, it was essential that all samples should be corrected for this parameter. Fig. 6 in the results section presents two alternative presentations of data showing this variation.

The method of white cell removal used was an adaptation of the technique described in detail by Lingard (1974a). In this a barrier medium of relative density (RD) 1·09 is prepared from di-n-butyl (RD 1·042) and dimethyl phthalate (RD 1·189), the volumes used being 50 ml and 23·1 ml, respectively (Balentyne and Burford, 1960; Danon and Markovsky, 1964). This relative density of 1·09 is below that of red cells (Danon and Markovsky, 1964) and above that for all white cells (Graham et al., 1955). A volume of 1 ml of barrier medium is transferred to a plastic-capped tube and 6 ml of sample layered above it (Fig. 1). The tube is now spun at 1900 g for 15 min (centrifuge Gallenkamp CFD 400), and upon removal is seen to have formed three layers: the packed
red cells at the base, the barrier medium with white cells at its surface and the plasma with other low-density debris at the top (Lingard, 1974a). The major portion of the plasma is now removed with a Pasteur pipette and further prepared by filtering through saline-soaked millipore PTFE filters (FALP 02500) of 0.3 μm pore diameter, held in Swinnex-13 holders (SXHA 013 OS). Next, the barrier medium is removed to waste, leaving the red cells, which are then mixed for 10 min in the filtered plasma at 25 per cent haematocrit. This concentration provides a realistic value, 50 cells per μm² pass through each pore, but is not so high as to interfere with the filter flow rate (Lingard, 1974b). The approximate pore flow velocity is 1.3 mm s⁻¹, compared with a representative capillary flow velocity range of 0.2-1.5 mm s⁻¹ (Caro et al., 1978).

The technique of white cell removal was 92 ± 1 per cent efficient in one pass as assessed by Coulter counter analysis, the whole preparation process for six samples taking 50 min. After preparation the red cell suspension gave repeatable results in the filtration system over a period greater than 75 min, sufficient time to complete all the tests.

With regard to the effect of the phthalate esters on red cells, it was assumed from the work of Danon and Markovsky (1964) and Lingard (1974a) that the method had no effect on red cell fragility or filterability. Under the microscope the appearance of red cells which had been subjected to the separation process was normal.

Initial work evaluating the filtration system was carried out using dog blood taken into heparin solution, as for human subjects, from an indwelling arterial catheter. This blood was processed identically.

2.2 Description of the filtration apparatus

The filtration apparatus is shown in Fig. 2. It is a development of the devices described by Reid et al. (1976) and Schmid-Schönbein et al. (1973). A pulsation-free 200 cm H₂O pressure is produced in the 201 capacitor by a Hy-flo minipump and led to the Perspex filtration unit. The 5 μm nuclepore filter (N 500 CPR 01900) is positioned as shown supported by a small cross-wire, with the 0.3 ml blood sample added above, giving a standard gravitational head of 5 mm at the start of each run. The diameter of the flow chamber is 12.5 mm, the intention being to reduce non-linearities produced by gravitational head and surface tension, in contrast to Barnes et al. (1977), Drummond et al. (1980) and Reid et al. (1976), where the diameter is about 5 mm. Using a pore density of 4 × 10⁶ cm⁻² and a flow area of 1.26 cm² the number of pores is approximately 5 × 10⁵. Easy access to the filtration unit is provided by quick-release clips. Fluid is prevented from flowing through under gravity above by a back pressure of 4 cm H₂O obtained from the minipump.

When valve A is closed, cutting off the back pressure, and B opened the lower chamber temporarily vents to atmosphere. Valves C and D are then opened with vent B now closed to start the flow, the driving pressure being monitored by a pressure transducer (Ether UPIT/C, 25 cm H₂O working maximum) and a digital voltmeter. As air is displaced from the collecting chamber by the suspension flow it transfers water via a syphon to the water balance (Buckley et al., 1979). This consists of a small 3 ml pan attached to a sensitive force transducer (Grass FT03C), the signal passing via an amplifier (Device MX2) to a chart recorder (Bryans 27000). The overall gain is 10⁷, giving a sensitivity of 20 μl cm⁻¹ on the trace.

2.3 Points of technique

Fig. 3 shows the form of the traces for saline and the red cell suspension of 25 per cent haematocrit. Traces 1 and 2 are...