Technical methods

A simple method for measuring erythrocyte deformability

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To pass through the microcirculation red blood cells (RBCs) need to undergo considerable deformation. The increased rigidity of older cells is probably the main cause of their selective trapping by the spleen; and indirect evidence suggests that impaired deformability may be a critical factor in causing diminished peripheral blood flow (Chien *et al*, 1967; Schmid-Schönbein *et al*, 1969; Weed, 1970). Several techniques have recently been developed to measure this property of the RBC, but so far none has achieved wide acceptance. The method described here is comparatively simple and has given reproducible results. It is a modification of the technique evolved by Schmid-Schönbein and his colleagues in 1973, but it uses whole blood instead of cells resuspended in artificial media.

Received for publication 30 March 1976

Principle

Under standard conditions whole blood is passed through a membrane filter using a negative pressure of 20 cm water. The speed of flow is determined largely by the deformability of individual RBCs, as the pore diameter (5 microns) is less than that of the red cell.

Apparatus

Figure 1 is a schematic representation of the apparatus. A simple hydrostatic mechanism is used for applying a known pressure gradient across the membrane filter. This suction mechanism is connected to a vacuum reservoir which, in turn, communicates with a filtrate reservoir. A syringe and membrane-holder containing the specimen are attached to the filtrate reservoir. All connections are made by glass and rubber tubings. A 25 ml

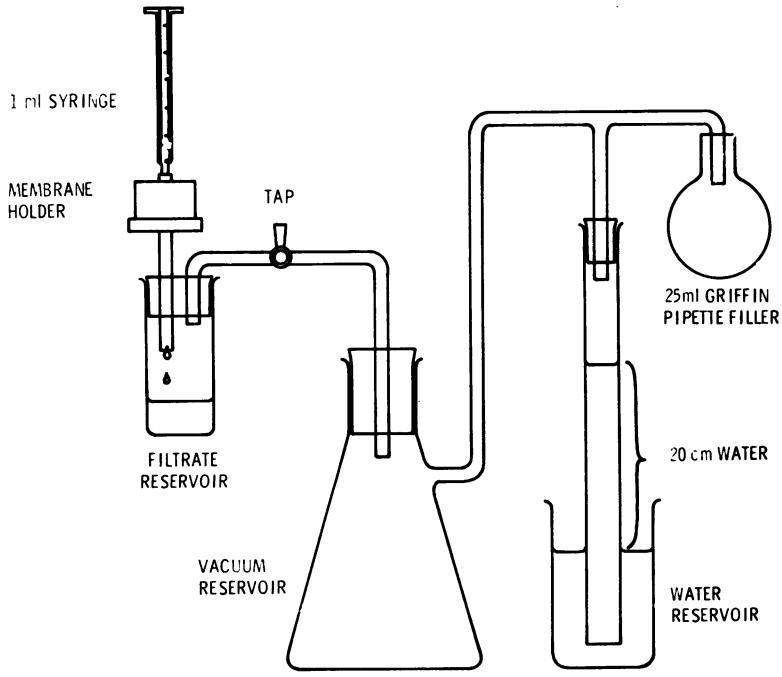

Fig 1 *A schematic representation of the apparatus.*

Griffin pipette filler fitted to the vacuum reservoir is used to evacuate the system by drawing up a 20 cm column of water from a second reservoir. The syringe and membrane-holder can easily be fitted to and disconnected from the reservoir which collects the filtrate.

Each membrane-holder has three parts: a clamp ring for positive locking; a cap with a female Luer slip-fit inlet and recess for easy filter loading; and a base with a support grid and a male Luer slip-fit outlet connection (fig 2). The assembled holder has a dead space volume of 0·34 ml.

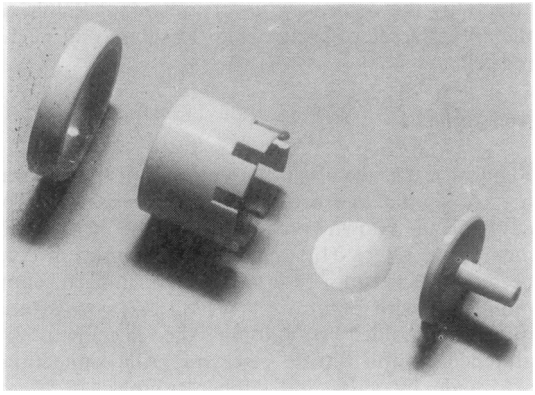

Fig 2 *Exploded view of membrane-holder, showing from left to right: clamp ring, cap, membrane, and base with support grid.*

According to the manufacturer's specification the membrane filters have a normal pore diameter of 5 microns. Each membrane disc has a pore density of 4×10^5 pores per cm^2 with a thickness of 10 microns. Scanning electron microphotographs confirm these figures, the coefficient of variation in pore diameter from a number of filters being 5·4%.

Both the Nuclepore membrane holder (FH 013 00110) and filters (N500 CPR 013 00) are manufactured by General Electric Company, Pleasantown, California 94566, USA and supplied by Shandon Southern Instruments Limited, Camberley, Surrey, England.

Filtration procedure

The membrane holder is assembled by first placing the membrane filter on to the support grid of the base. The cap is then positioned over the base and pressed down until it snaps into place. The clamp ring is then secured over the cap until it is firmly seated over the fingers of the cap and the base of the holder.

Blood is withdrawn from an arm vein, with minimum venous occlusion, into a clean disposable plastic syringe. A 5 ml sample is anticoagulated with 2 mg (± 10%) ethylenediaminotetra-acetic acid (EDTA).

The assembled membrane holder is filled with blood, using an Agryle Medicut Intravenous plastic cannula attached to a syringe. The filled holder is subsequently mounted on to the filtrate reservoir by way of the male Luer slip-fit outlet, and a 1 ml graduated plastic syringe, without its plunger, is inserted. The graduated syringe is also filled with blood, to the 1 ml mark, using the plastic cannula. The filtration is begun when the perfusion pressure is applied by opening the tap between the filtrate reservoir and the vacuum. A stop-watch is started at the same time.

Although the filtration system is made virtually airtight, there is invariably a pressure drop of 1 to 2 mm of water during the course of the filtration. This pressure drop can be prevented, however, by constant attention to the suction device.

Deformability index

RBC deformability is recorded either as the flow time or, in the case of abnormal blood, as the volume of blood filtered in one minute. In either case the results are expressed as the volume of red blood cells (V_{RBC}) filtered per minute, which we define as the 'deformability index'. By expressing the results in this way we take account of the packed cell volume, measured by microhaematocrit, but disregard the time taken for plasma to flow. Readings are taken in triplicate on each subject's blood, using a new filter for each measurement. All deformability studies are performed at room temperature (22-25°C in our laboratory) and within two hours of venepuncture.

Results

The coefficient of variation for tests from the same sample was 10·0% for individual observations, while for the same subject's blood measured on five consecutive days it was 13·0% for individual observations. This fell to 4·4% if the means of triplicate observations were used. Delay for more than three hours in measuring a sample is associated with a gradual decrease in V_{RBC}. Therefore blood samples are allowed to equilibrate to room temperature and measurements are made within two hours of withdrawal.

Figure 3 shows a Scanning electron microphotograph of the Nuclepore membrane filter as viewed

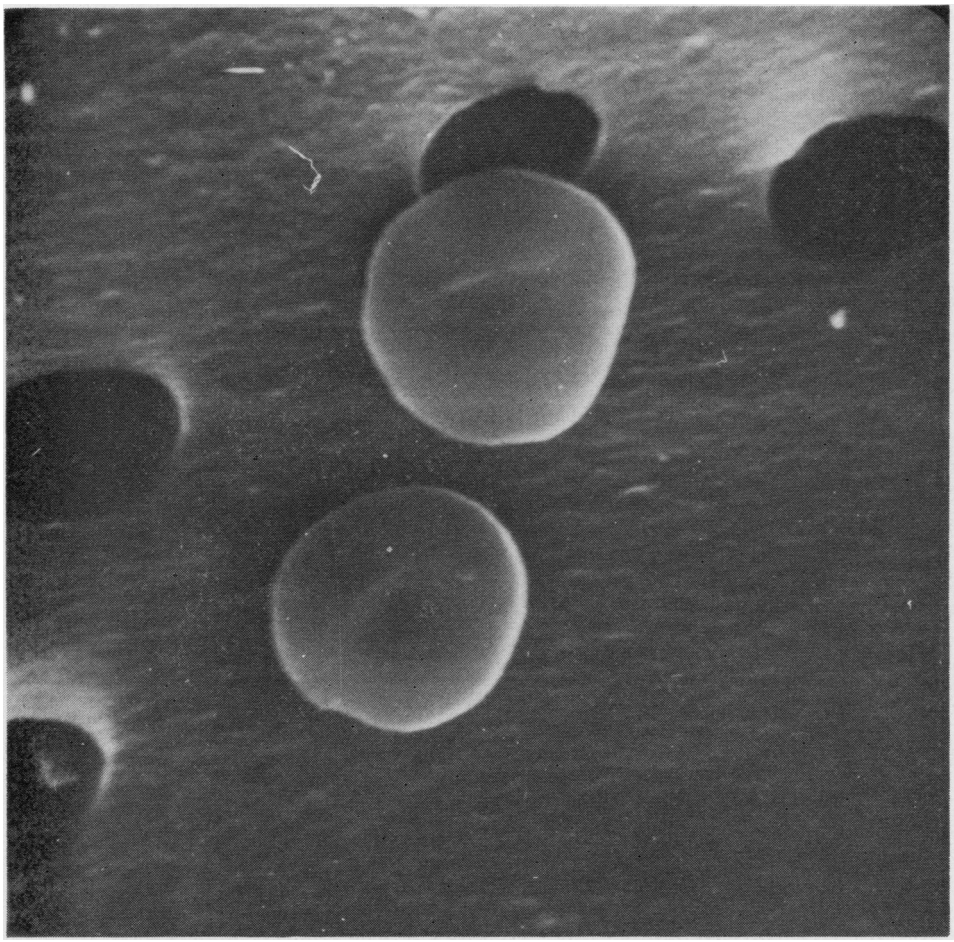

Fig 3 *A scanning electron microphotograph of Nuclepore filter as viewed from above, showing the relationship of RBCs to pore size (× 7·20 K).*

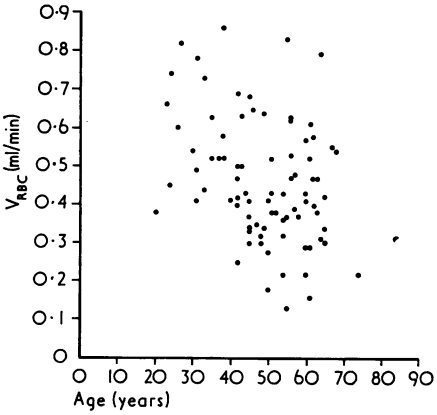

Fig 4 *Erythrocyte deformability in relation to age (r = −0·36).*

from above. The relationship of two RBCs, from a normal subject, to pore size can alsobe seen.

Figure 4 shows V_{RBC} as a function of age in 84 normal subjects (51 males and 33 females) with an age range of 23 to 84 years. Over this range the correlation coefficient (r) between RBC deformability and age was −0·36. There was no significant difference between males and females.

From studies using red cells resuspended in their native plasma at varying concentrations it was found that V_{RBC} is constant between a PCV of 20 and 70%, suggesting that in this range V_{RBC} is independent of PCV.

Discussion

The relative merits of different experimental approaches to RBC deformability clearly depend on the principal objective of the study. If the main

objective is to explore cell deformability as a physical characteristic in its own right, techniques using cells resuspended in artificial media (Gregersen *et al*, 1967; Weed *et al*, 1969; Forman *et al*, 1973; Schmid-Schönbein *et al*, 1973) or packed cell preparations (Jacobs, 1963; Dintenfass, 1965) can yield valuable information. However, as the physical properties of the red cells are almost certainly influenced by the surrounding plasma, we feel that the measurement of RBC deformability in whole blood is more clinically relevant. This appears to be borne out by preliminary studies of RBC deformability in patients with peripheral circulatory insufficiency (Reid *et al*, 1976).

In trying to reproduce conditions which prevail in the microcirculation the characteristics of the filter used are obviously critical. Several comparative studies of filtering devices have been published (Nicolau *et al*, 1961; Gregersen *et al*, 1967; Forman *et al*, 1973; Rosenmund *et al*, 1975) and there seems to be general agreement that Nuclepore filters give results which are both sensitive and reproducible.

References

Chien, S., Usami, S., Dellenback, R. J., and Gregersen, M. I. (1967). Blood viscosity. Influence of erythrocyte deformation. *Science*, **157**, 827.

Dintenfass, L. (1965). Viscosity of the packed red and white blood cells. *Exp. molec. Path.*, **4**, 597-605.

Forman, S., Bischel, M., and Hochstein, P. (1973). Erythrocyte deformability in uremic hemodialysed patients. *Ann. intern. Med.*, **79**, 841-843.

Gregersen, M. I., Bryant, C. A., Hammerle, W. E., Usami, S., and Chien, S. (1967). Flow characteristics of human erythrocytes through polycarbonate sieves. *Science*, **157**, 825-827.

Jacobs, H. R. (1963). The viscosity of red cell packs. *Biorheology*, **1**, 129-138.

Nicolau, C. T., Teitil, P., Fotino, M., Butoianu, E., and Taigar, S. (1961). Alteration of erythrocyte plasticity in blood disease. (A three year clinical experience with the erythrocyte filterability test (EFT)). *Sangre (Barcelona)*. **9**, 282-288.

Reid, H. L., Dormandy, J. A., Barnes, A. J., Lock, P. J., and Dormandy, T. L. (1976). Impaired red cell deformability in peripheral vascular disease. *Lancet*, **1**, 666-668.

Rosenmund, A., Binswanger, U., and Straub, P. W. (1975). Oxidative injury to erythrocytes, cell rigidity, and splenic hemolysis in hemodialyzed uremic patients. *Ann. intern, Med.*, **82**, 460-465.

Schmid-Schönbein, H., Weiss, J., and Ludwig, H. (1973). A simple method for measuring red cell deformability in models of the microcirculation. *Blut*, **26**, 369-379.

Schmid-Schönbein, H., Wells, R., and Goldstone, J. (1969). Influence of deformability of human red cells upon blood viscosity. *Circulat. Res.*, **25**, 131.

Weed, R. I. (1970). The importance of erythrocyte deformability. *Amer. J. Med.*, **49**, 147-150.

Weed, R. I., LaCelle, P. L., and Merrill, W. E. (1969). Metabolic dependence of red cell membrane deformability. *J. clin. Invest.*, **48**, 795.

A rapid microbiological procedure using *Bacillus stearothermophilus* for the assay of antibacterial drugs

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The essential criteria for clinical assay procedures have been well summarized elsewhere (Phillips *et al*, 1974) as reasonable accuracy, simplicity, and speed both in technician time and in result. In 1971 Wahlig and Haemeister reported a method which seemed to combine these qualities with those of extreme sensitivity and the use of extremely small volumes of body fluids; this method was originally intended for gentamicin assays, and the present paper outlines the ways in which this idea has been developed. An unusual feature is that the assay indicator organism is *Bacillus stearothermophilus*, heavy inocula of which when incubated at about 60°C reach visible growth in three hours on a plate. One of us (HW) has developed the method in kit form[1] with premeasured, dehydrated medium and gentamicin standards and a dried *B. stearothermophilus* spore suspension; the procedure was used under clinical conditions by Spring *et al* (1971), who found it to be highly satisfactory and to give excellent correlation with a standard microbiological method. The other (RJH) has adapted the principle as a daily routine test for amino-hexose and also for several β-lactam antibiotics as well as for erythromycin, chloramphenicol, lincomycin, chlorolincomycin, fusidic acid, and the tetracyclines. The method has been used for clinical asays of each of these drugs.

Received for publication 10 May 1976

[1]Refobacin-Test, Art. No. 11400, E. Merck, Darmstadt, Germany