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Flow Characteristics of Human Erythrocytes through Polycarbonate Sieves

Abstract. *We used polycarbonate sieves with uniform cylindrical pores (2.4 to 6.8 microns in diameter) to filter suspensions of human erythrocytes (mean major diameter is 7.2 microns) in Eagle-albumin solution. With 6.8-micron sieves the pressure-flow curves are convexed to the pressure-axis at low pressures and become linear with high pressures. With 4.5-micron sieves, however, the pressure-flow relationship is linear throughout the range of study. In both types of sieves, flow rate is reduced progressively with increasing concentration of red blood cells (RBC) over a range of 0.5 to 95 percent. The resistance to flow of RBC suspensions is higher in 4.5-micron than in 6.8-micron pores. With filter pore diameters of 3.0 microns or more, the RBC concentration in the filtrate was 100 percent of that in the solution being filtered, but only 70 percent with 2.4-micron pores. The observed critical pore diameter for 100 percent cell transmission agrees with theoretical calculation based on the assumption that the RBC membrane is deformable but nonextensible. The importance of cell deformation in the passage of RBC's through small pores is shown by the inability of RBC hardened in acetaldehyde to pass pores with 6.8-micron diameter.*

Our data, presented here in three papers, show that the departure of blood from Newtonian behavior depends on two mechanisms: (i) erythrocyte deformation, which preserves the fluidity of blood at normal and high cell concentrations and also enables red cells to squeeze through the smallest capillaries, and (ii) erythrocyte aggregation (rouleaux formation), which accounts for the striking shear-rate dependence of viscosity at near-zero flow conditions. In this paper we examine RBC deformation by sieving tests; we have also compared the viscometric behavior of deformable and hardened (rigid) RBC (*1*) and have shown the influence of RBC aggregation (rouleaux formation) on blood viscosity (*2*).

Fleischer *et al.* (*3*) recently described a new technical development of polycarbonate filters (sieves) with cylindrical pores of measurable diameter and known length. We performed experiments to see if these sieves could be used to obtain quantitative data on (i) the rheological behavior (pressure-flow relations) of erythrocyte (RBC) suspensions and (ii) RBC deformation in passing through cylindrical pores with diameters comparable to those of vessels in the microcirculation. We used only suspensions of washed RBC's to exclude the complicating effects of other formed elements (*4*) and of various cell-protein interactions (*5*) because our aim was to examine the properties of the sieves in relation to the flow characteristics of normal RBC's *per se*.

Human blood was washed three times with Eagle-albumin solution (*6*) and then suspended in it. Counts of RBC's were made with the Coulter electronic counter, and the cell percentage (H) was determined by the microhematocrit method (5 minutes centrifugation at 15,000*g*). From these results we calculated the mean corpuscular volume (MCV) of the washed RBC's, which agreed with that of the fresh blood. Microscopic observations showed normal biconcave RBC's after washing with Eagle-albumin solution. Suspensions of desired RBC concentrations were prepared immediately in the Eagle-albumin solution, and the sieving experiments were performed as soon as practicable.

The polycarbonate sieves used had various mean pore diameters (Fig. 1). The sieve and its metal supporting screen were clamped in a filter funnel with an effective filter area of 8.0 cm^2. The funnel stem was inserted through a rubber cork used to stopper a collecting cylinder. By setting the negative pressure in the cylinder, we could keep the filtration pressure (ranging from 0.5 to 20 cm of H_2O) constant.

All filtration experiments were done at 22° to 25°C. We wet each new filter with Ringer solution (*7*) before its installation and calibrated it immediately by timing the flow rate of the Ringer or Eagle-albumin solutions, both of which gave the same results. In filtering the RBC suspensions, we spread a measured volume (1 or 2 ml) of the mixed suspension uniformly over the filter and determined the passage time by observing the filter. The passage of successive samples of RBC suspensions through the sieves (especially those with pore diameters less than 5 μ) caused progressive occlusion of the pores and decrement in flow rate. Repeated washing of both sides of the filter under suction with Ringer solution usually cleared the occluded pores, as indicated by recalibration with Ringer solution. When this failed, the sieve was soaked in 6*N* nitric acid overnight, and new filter with comparable characteristics was used to continue the experiment.

We determined the rate of flow decrement of RBC suspensions caused by pore occlusion by timing the passage of discrete volumes in rapid succession at a constant filtration pressure. Figure 2 is a semilogarithmic plot of the flow rate and the cumulative volume passed (V) in one test. The data can be fitted by a simple exponential function:

$$\text{Flow} = \dot{V}_0 e^{-\lambda V}$$

where $\dot{V}_0$ (intercept on the flow axis) is the extrapolated initial flow rate in milliliters per second and λ is the

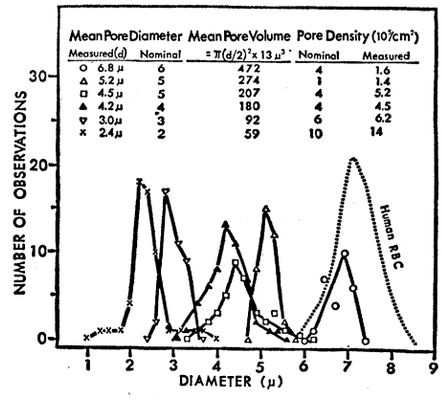

Fig. 1. Characteristics of polycarbonate sieves. The nominal values are data supplied by General Electric Atomic Products Division, Vallecitos Atomic Laboratories, Pleasanton, Calif. The filter thickness is 10 to 12 μ for all sieves. Because the pores are generally not perpendicular to the filter surface, we assume the mean pore length to be 13 μ in calculating the mean pore volume. The measured values for pore diameters and pore density were obtained by direct microscopic observations or from photomicrographs of the sieves. Solid lines represent the distribution curves for measured pore diamters of various sieves. Dotted line is the Price-Jones distribution curve for major diameter of normal human erythrocytes.

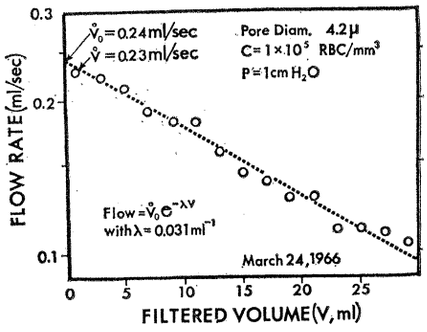

Fig. 2. Semilogarithmic plot showing flow decrement with successive filtration of 2-ml samples of RBC suspension.

rate of flow decrement (in milliliters^{-1}). With a constant filtration pressure (P), there is a strong positive correlation between λ and H; and at a given H, λ is inversely related to P. That is, the flow decrement is most pronounced when suspensions with high RBC concentration are filtered at low pressure.

The extrapolated initial flow rate $\dot{V}_0$ is nearly the same as the flow rate for the first 2-ml sample ($\dot{V}$) (Fig. 2). Hence, for obtaining pressure-flow data, we simply measured $\dot{V}$ at different pressures in rapid succession, using a cleaned or fresh filter for each test. Such data were obtained for suspensions with H ranging from 0.5 to 95 percent RBC. Even with H equal to 0.5 percent, the flow at any pressure was slower than that of the Ringer or Eagle-albumin solution (H = 0 percent). This reduction in flow became increasingly pronounced as H was elevated. The pressure-flow curves at low P are convexed toward the P-axis, but then become essentially linear at high filtration pressures. Downward extrapolation of the linear segments of these curves results in a common nodal point below zero-flow. These pressure-flow relationships for 6.8-μ pores are qualitatively the same as those observed for capillary tubes with a radius of 185 μ (*8*).

In contrast, the pressure-flow curves obtained on 4.5-μ sieves are linear throughout the range of measurement, and downward extrapolations of these lines go through the origin (Fig. 3B). Therefore, for a given H, the resistance to flow ($P/\dot{V}$) in a 4.5-μ sieve is constant. The resistances encountered by the RBC suspensions are divided by the resistance for Ringer solution filtered through the same filter at the same pressure; the resulting relative resistances are plotted in Fig. 3C. In 6.8-μ sieves, as a corollary of the nonlinearity of the pressure-flow curves, the relative resistances are dependent on the pressure and rise at low pressures (Fig. 3C). For a given H, the relative resistance encountered by the RBC suspensions through a 6.8-μ sieve is markedly lower than that through a 4.5-μ sieve, but the difference becomes progressively smaller as P is reduced.

Figure 3, A and B, includes ordinate scales for flow through each pore, which we calculated by dividing the total flow by the product of total filtration area (A) and pore density (σ). A comparison between the two types of sieves at a given high pressure (for example, 10 cm of H_2O) shows that the flow of RBC suspensions through a 6.8-μ pore is approximately nine times faster than that through a 4.5-μ pore, a difference greater than that expected from the flow of a simple fluid.

With filters of 3-μ mean pore diameter or larger, the RBC count in the filtrate was, within limits of error, the same as that in the original suspension. However, when a filter with a mean pore diameter of 2.4 μ was used, the RBC count in the filtrate was only 66 to 74 percent of the RBC count in the original suspension. These results fall in line with the following theoretical calculations of the smallest pore diameter of a given length (for example, 13 μ) which the RBC can traverse without stretching its membrane. On the basis of the observation by Rand (*9*) we assume that at the entrance to a small cylindrical pore the optimum shape for an RBC without membrane stretching is that shown in Fig. 4 (upper left corner). The volume is here distributed in three parts: a spherical segment with diameter x above the filter pore; a cylinder with a diameter d inside the pore; and below this, a hemisphere also with diameter d. We have calculated the x values for an RBC having a cell volume of 100 μ^3 and a flexible but nonextensible membrane with surface area of 150 μ^2 when the RBC enters sieves of different pore diameters (d). As d increases, a larger percent of the RBC volume enters the pore without stretching the cell membrane, and x becomes reduced. With a large enough pore, the value of x can be reduced to equal d (Fig. 4, upper right corner), and the RBC can pass through the pore without increasing its surface area. Our calculations show that this occurs when d reaches 2.84 μ (Fig. 4). That is, when the filter pore diameter is greater than 2.84 μ, an RBC with a volume of 100 μ^3 and a surface area of 150 μ^2 can pass with-

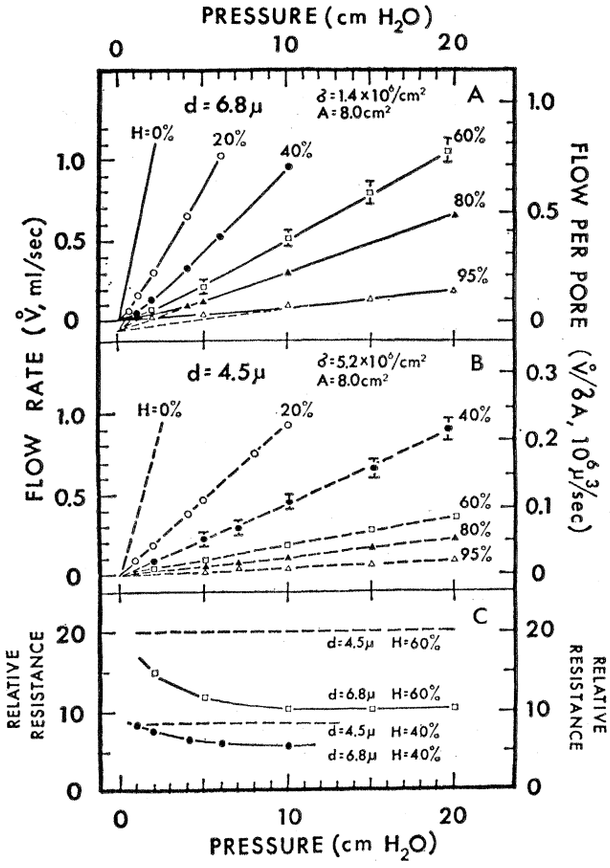

Fig. 3 Pressure-flow relationships of 0 to 95 percent human RBC suspensions through 6.8-μ (A) and 4.5-μ sieves (B). The ordinates at left give total flow through the sieve; those at right give the average flow through each pore. Results are mean values from four to eight experiments. The standard errors of the mean are given for H equal to 60 percent in A and H equal to 40 percent in B. From the results in A and B on 40 and 60 percent RBC suspensions, relative resistance (flow of Ringer solution/flow of suspension) values are calculated (C). In 4.5-μ sieves, the relative resistances of RBC suspensions are essentially constant. The relative resistances through 6.8-μ sieves are lower at high pressures.

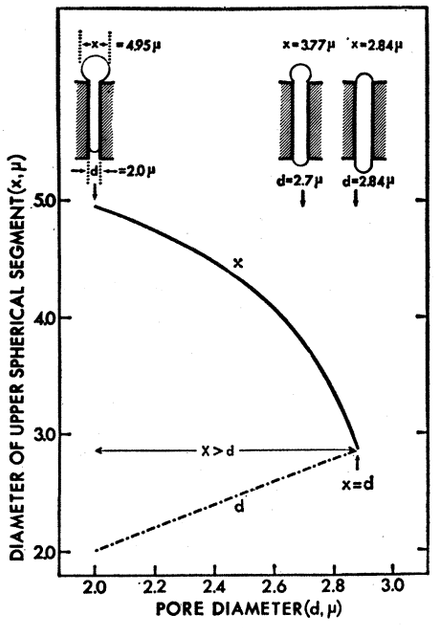

Fig. 4. Theoretical calculation of critical pore diameter through which an RBC with a volume of 100 μ^3 and a surface area of 150 μ^2 can pass without membrane stretching. The diameter (x) of the upper spherical segment of the RBC is reduced as the filter pore diameter (d) is increased. The curve for x intersects the dotted line d when the RBC can pass through the pore without tensile strain. Changes in x at three selected d values are shown in the upper part of the figure.

out tensile strain. This is in agreement with our experimental findings and with the results reported by Burton (*10*).

The ability of RBC's with a mean major diameter of over 7 μ (Fig. 1) to pass through smaller pores obviously necessitates cell deformation (Fig. 4). In this connection, it should be noted that RBC's hardened with acetaldehyde (*1*), although having normal size and shape, cannot be recovered in the filtrate even when sieves with 6.8-μ pores are used.

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Blood Viscosity: Influence of Erythrocyte Deformation

Abstract. *Suspensions of canine and human erythocytes hardened with acetaldehyde differ from the suspensions of normal erythrocytes with respect to their rheological behavior. Normal erythrocytes can be packed by centrifugation so that the sediment volume is nearly 100 percent cells, but the hardened erythrocytes (RBC's) can be packed only to approximately 60 percent cells. At the same cell percentage the viscosity of the hardened RBC suspension is higher than that of the suspension of normal erythocytes. An increase in shear stress deforms the normal erythocytes and lowers the suspension viscosity, but has no influence on the viscosity of the hardened cell suspension. In blood with high cell percentages, the shear deformation of normal RBC's plays an important role in reducing viscosity and facilitating flow at high shear stresses.*

There is ample evidence that normal erythrocytes (RBC's) can be deformed (*1*, *2*), but the role of RBC deformation in determining the flow properties of the blood has not been clearly established. In our experiments, we compared the viscometric behavior of suspensions of hardened RBC's and normal RBC's in an attempt to analyze the relation between RBC deformability and suspension viscosity.

Human and canine RBC's were washed with 0.9 percent NaCl solution and hardened in a 2 percent (weight to volume) acetaldehyde solution in 0.9 percent NaCl (*p*H buffered to 7.4) (*3*). After 1 month of hardening, the RBC's were washed with and then suspended in 0.9 percent NaCl or distilled water.

During the first week of the hardening process, the RBC suspension in acetaldehyde showed a progressive increase in the hematocrit reading as obtained by centrifugal packing. Since the RBC size determined by microscopic observation as well as with an electronic particle counter was unchanged, the rise in hematocrit suggested a reduction in the completeness of centrifugal packing rather than RBC swelling. The degree of trapping of the fluid medium in the packed cell column was determined by a dilution technique (*4*) with radioactive macromolecules (albumin-I^{131}, dextran-C^{14}, or polyvinylpyrrolidone-C^{14}). After centrifugation at 15,000*g* for 5 minutes or at 1,500*g* for 30 minutes, the volume fraction of the packed cell column occupied by hardened RBC's averaged only 0.60 (standard error of mean = 0.01); that is, 0.40 of the column consisted of trapped fluid. Centrifugation at 15,000*g* for 30 minutes increased the volume fraction occupied by hardened RBC's only slightly (0.62). In contrast, centrifugation of normal RBC suspensions under these conditions gave packed columns with RBC's occupying 0.95 to 0.97 of the volume. The value of 60 percent packing for hardened RBC's agrees closely with that calculated on a theoretical basis (*5*).

Suspensions of normal and hardened RBC's were made to contain various

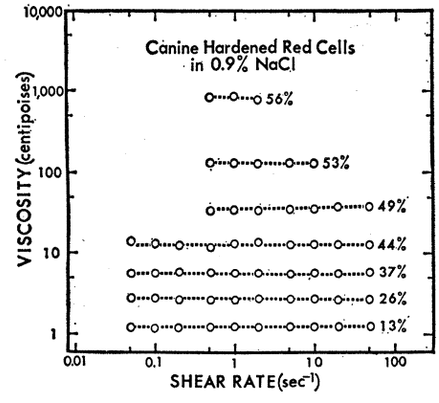

Fig. 1. A log-log plot of the relationship between viscosity and shear rate for suspensions of hardened canine RBC's in 0.9 percent NaCl. The cell percentages for the suspensions are shown for each curve. The viscosity values are essentially independent of the shear rate.