THE PHYSICS OF BLOOD FLOW IN CAPILLARIES

III. The Pressure Required to Deform Erythrocytes in Acid-Citrate-Dextrose

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ABSTRACT Two previous communications dealt with the nature of the motion and the resistance to flow in capillary blood flow. In this final paper measurements are reported of the pressure required to force mammalian erythrocytes through pores having a diameter less than the cells. The cells, from fresh human or dog blood, were resuspended in acid citrate dextrose solutions. The final suspensions (about 1,000 times more dilute than whole blood) were immediately emptied into a millipore filter apparatus and the rate of filtration was measured. Filters having pore diameters of 5.0 and 3.0 microns were employed. The cellular concentration of samples of the original suspensions and of the filtrate was determined. It was observed that the rate of filtration decreased rapidly initially and then became constant. In the 'steady state' the cellular concentration of samples of the filtrate was found to be approximately equal to that of the original suspension. A simple theory is presented which adequately describes the flow of a suspension through such filters. It is concluded that mammalian erythrocytes, particularly human and dog cells, will pass steadily, without hemolysis, through pores 5.0 or 3.0 microns in diameter under pressures of 4 cm of water or less.

1. INTRODUCTION

In capillary blood flow the erythrocytes (red cells) are commonly observed to move along in "trains" with the cells separated from one another by segments of plasma (bolus flow). The first paper (1) of this series dealt with the peculiar motion of the plasma in bolus flow and the effect of this motion on the equilibration of gases in the capillary. The second paper (2) dealt with the fluid resistance associated with capillary blood flow. It was noted that the pressure drop (or resistance) associated with the flow of blood within the capillary arose in three ways; *viz.*, viscous resistance in the plasma, frictional resistance between the capillary wall and the red cell, and viscous resistance within the red cell when the cell contents are sheared. However, when bolus flow occurs in the capillaries a fourth factor contributes to

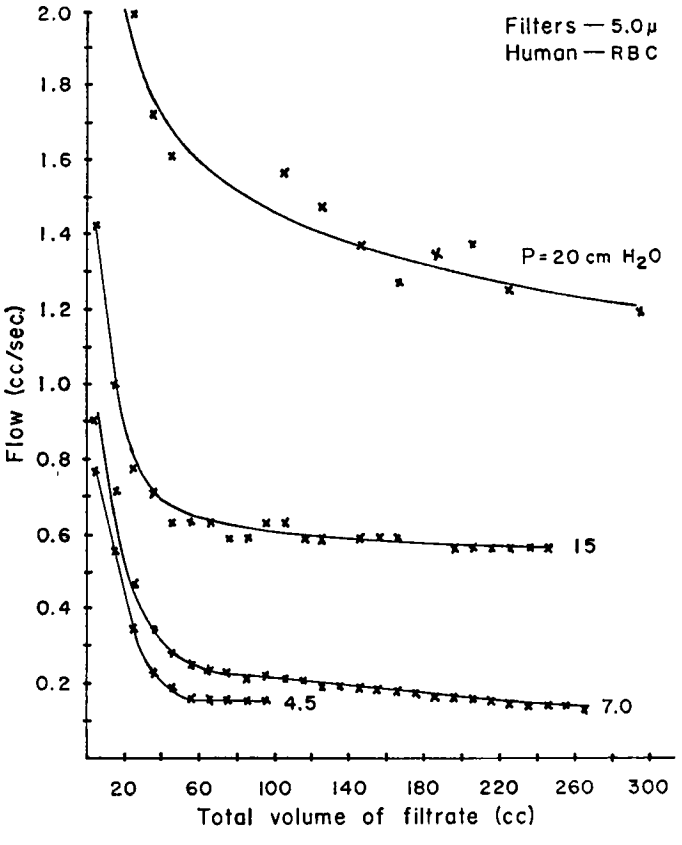

FIGURE 1 The flow of dilute suspensions of (approximately 3500 cells/mm³) red blood cells through 5.0 micron millipore filters.

the total pressure drop, namely that required to deform a red cell sufficiently to enable it to enter a capillary of smaller diameter. Krogh (3) has remarked upon the small pressures required to deform mammalian red cells but no values for this pressure appear to have been published heretofore. This third paper of the series presents data which determines at least an upper limit to the pressures required.

2. METHOD

The principle of these experiments was to overlay a dilute suspension of mammalian red cells on a millipore filter[1] and observe the production of filtrate. The depth of the suspension is a direct measure of the hydrostatic pressure tending to force cells through the filter. The rate at which filtrate appears may be measured directly. Fur-

[1] Millipore Filter Corporation, Bedford, Mass. The filters employed in these experiments had, according to the manufacturer's specifications, pore diameters of 5.0 ± 1.2 microns and 3.0 ± 0.9 microns.

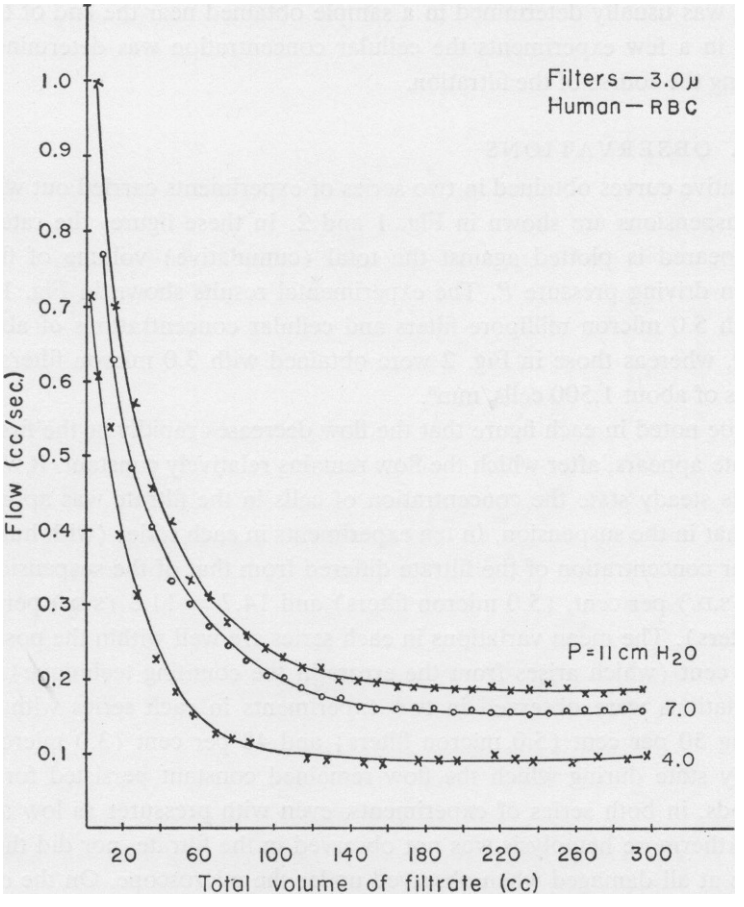

FIGURE 2 The flow of dilute suspensions (approximately 1100 cells/mm³) of human red blood cells through 3.0 micron millipore filters.

thermore the cellular concentration of the filtrate my be readily determined (cell counting) and compared with the cellular concentration of the original suspension.

The procedure was to obtain fresh blood from humans, or from dogs, and to centrifuge the blood. The cells obtained after decanting the plasma were resuspended, the human cells in acid citrate–dextrose (at 35°C), the dog cells in heparinized Ringer's solution. The final suspensions were roughly two thousand times more dilute than whole blood. When the suspension was obtained it was immediately emptied into the reservoir of the filter apparatus. The time interval required for each successive 10 cc fraction of the filtrate to appear was determined. The level of the suspension in the reservoir was maintained, and sedimentation was prevented by adding more fluid, with stirring, at short intervals as the level fell. The cellular concentrations of the suspension and of the filtrate were determined. The latter con-

centration was usually determined in a sample obtained near the end of the experiment, but in a few experiments the cellular concentration was determined several times during the course of the filtration.

3. OBSERVATIONS

Representative curves obtained in two series of experiments carried out with human red cell suspensions are shown in Fig. 1 and 2. In these figures the rate at which filtrate appeared is plotted against the total (cumulative) volume of filtrate, for some given driving pressure P. The experimental results shown in Fig. 1 were obtained with 5.0 micron millipore filters and cellular concentrations of about 3,000 cells/mm^3, whereas those in Fig. 2 were obtained with 3.0 micron filters and concentrations of about 1,500 cells/mm^3.

It may be noted in each figure that the flow decreases rapidly as the first 50 cc or so of filtrate appears, after which the flow remains relatively constant. It was verified that in this steady state the concentration of cells in the filtrate was approximately equal to that in the suspension. In ten experiments in each series (with human cells) the cellular concentration of the filtrate differed from that of the suspension by only 8 ± 6.5 (S.D.) per cent, (5.0 micron filters) and 14.7 ± 11.3 (S.D.) per cent (3.0 micron filters). The mean variations in each series are well within the possible error of 20 per cent (which arises from the errors in the counting technique). However larger variations were observed in two experiments in each series with dog cells, these being 30 per cent (5.0 micron filters) and 48 per cent (3.0 micron filters). The steady state during which the flow remained constant persisted for relatively long periods, in both series of experiments, even with pressures as low as 4 cm of water. Furthermore hemolysis was not observed in the filtrate, nor did the cells appear to be at all damaged when observed under the microscope. On the other hand if a small amount of blood was deliberately hemolysed, then diluted and poured into the reservoir, it was observed that the filter rapidly became plugged.

The results obtained in four experiments in which the pressure was lowered to one cm of water after the steady state was obtained (at a higher pressure) are summarized in Table I.

The manner in which the concentration of cells in the filtrate increases as the

TABLE I

Experiment	Initial pressure	Cellular concentration	
		Suspension	Filtrate
	cm water	*cells/mm^3*	*cells/mm^3*
1	4	1110	1190
2	4	860	720
3	4	1027	1090
4	7	1250	1240

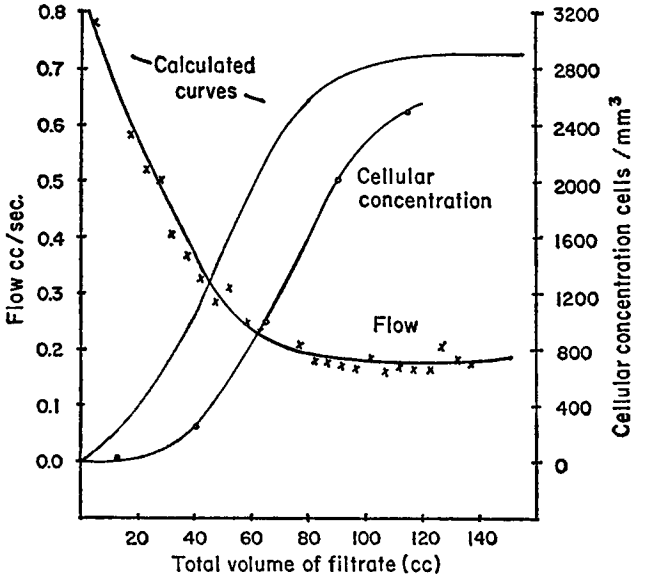

FIGURE 3 A suspension having a cellular concentration of 2900 cells/mm³, at a pressure of 7 cm of water, was filtered through a 3 micron millipore filter. The cellular concentration (circles) was determined in successive 25 cc fractions of the filtrate. For a discussion of the calculated curves see Appendix I.

flow decreases is illustrated in Fig. 3. The mean cellular concentration (circles on graph) was determined for each successive 25 cc. fraction of filtrate.

4. DISCUSSION AND CONCLUSIONS

Mammalian red cells, in particular those of humans and dogs, will pass steadily through pores of 5.0 or 3.0 microns diameter for relatively long periods, under pressures of 4 cm of water. In four experiments (see Table I) in which the pressure was lowered (after the steady state was reached) to 1 cm of water and then allowed to drop gradually to zero, essentially 100 per cent of the cells still passed through the filter.

The conclusion that red cells pass through such minute pores, under low pressures, is questionable unless it is established that the pore size distribution is indeed as restricted as the manufacturer states. If a few large pores are present it could be argued that the cells pass only through the large ones. However the narrowness of the pore size distribution in filters of this type has been verified independently by Honold and Skau (4) using the mercury intrusion method. Furthermore the agreement between our calculated rate of decrease of flow (which is based in part upon the manufacturer's porosity data) and the observed rate of decrease of flow furnishes strong indirect evidence that the manufacturer's data is essentially correct.

A typical example of a calculated volume-flow curve and volume-concentration curve is shown in Fig. 3. In this figure the calculated volume-flow curve fits the data reasonably well. The difference between the calculated volume-concentration curve and the observed curve may be attributed to the fact, which is supported by visual observation, that the cells adhere to the underside of the filter. The discrepancy may be accounted for by a layer of cells over the filter less than 15 microns deep. The theory upon which these calculations are based is given in detail in Appendix I. In brief it is assumed that the filter can be treated as a large array of parallel channels of uniform bore,[2] through some increasing proportion of which cells and fluid flow (rather slowly), and through the remainder of which only fluid flows. The proportion of pores containing cells is assumed to reach a maximum in the steady state, and thereafter remain constant. The theory permits one to calculate the cellular concentration of a sample of filtrate. The curious result that the cellular concentration of a sample of filtrate may be higher than that of the original suspension arises because the volume of fluid (V_c) associated with each cell is much larger than the volume (V_p) of an individual pore (see Fig. 4). As he cell moves through a pore it "sweeps"

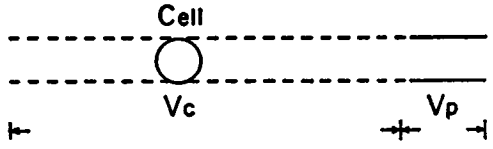

FIGURE 4 As a cell passes through a pore it carries with it a volume of fluid equal to that of a pore (V_p), which may be very much less than the volume per cell (V_c). The cellular concentration of a small sample collected at this time may thus be much higher than the concentration of the original suspension.

out a volume V_p, and thus the concentration in this particular sample is $1/V_p$, which evidently may be much larger than $1/V_c$. This argument may be extended to many pores in parallel. Of course in a very large sample the cellular concentration of the filtrate closely approaches that of the suspension.

In conclusion, it is stated that mammalian red cells may be deformed sufficiently to enable them to pass through pores 5 or 3 microns in diameter for relatively long periods under pressures of 4 cm of water or less. These pressures are less than the generally accepted values for the driving pressure available in the capillary circulation. On the other hand it is not believed that these experiments furnish reliable evidence on the viscous resistance to flow through capillaries *in vivo*. The calculated velocities through those pores containing cells (*cf.* Appendix I) are only about 1/500 of the values observed *in vivo*. Presumably the red cells adhere rather firmly

[2] The manufacturers represent the pores as strictly parallel straight tubes. We were most anxious to check this by an electron micrograph showing a section at right angles to the surface of a filter. However, all attempts to find a suitable embedding material which did not disintegrate the filter have so far failed.

to the filter material, in contradistinction to the case for the endothelial lining of capillaries (5).

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APPENDIX I

A. INTRODUCTION

The rate of filtration of a suspension may be formulated in a general way in terms of four parameters:

(*a*) initial flow (F_i)
(*b*) final flow (F_f)
(*c*) total number of pores (N)
(*d*) cellular concentration of suspension (C_s)

In the following analysis these parameters are employed to define the flow through individual pores and then to obtain equations for the rate of filtration.

B. EQUATIONS OF FLOW

Consider the initial flow (F_i) which is equal to the flow which would obtain with a cell-free fluid at the same driving pressure. The flow of liquid (F_l) through each individual pore is equal to:

$$F_l = F_i/N \tag{1}$$

The flow immediately begins to decrease from the initial value due to cells entering the pores of the filter. At any time t thereafter some proportion $N'(t)$ of the pores contain cells. The flow through a single pore assumed to contain one cell (see later) and liquid may be designated by F_c. The total flow $F(t)$ is the sum of the flows through those pores containing only liquid and those containing a cell.

$$i.e. \qquad F(t) = N'F_c + (N - N')F_l \tag{2}$$

Until the steady state is reached the proportion of pores containing a cell must equal the number of cells impinging upon the filter:

$$i.e. \qquad dN/dt = C_sF(t) \tag{3}$$

assuming that no cells adhere to the upper surface of the filter. If equation (2) is differ-

entiated with respect to time, the resultant equation may be employed to eliminate dN/dt from equation (3). Thus the flow of a suspension through a filter is described by the differential equation:

$$dF/dt = -C_c(F_i - F_c)F(t) \tag{4}$$

The complete solution to equation (4) is the sum of the steady state and transient terms:

$$i.e. \qquad F(t) = F_f + (F_i - F_f)e^{-yt} \tag{5}$$

$$\text{where} \quad y = C_c(F_i - F_c) \tag{6}$$

An expression for the volume, as a function of time, is obtained by integrating equation (5). It is possible to eliminate the time dependence from this expression by expressing the volume of filtrate as a function of the flow.

$$i.e. \qquad V(F) = \frac{1}{y}\left[(F_i - F) - F_f \ln\left(\frac{F - F_f}{F_i - F_f}\right)\right] \tag{7}$$

In order to evaluate equation (7) it is necessary to know Y, which in turn requires a knowledge of F_c. It is possible to evaluate F_c from a consideration of the steady state.

C. THE STEADY STATE

In the steady state, and after a sufficient time, the concentration of cells in a sample of the filtrate is known (empirically) to be equal to that of the suspension. Each cell in the suspension is associated, on the average, with a small volume (V_c) of liquid, given by:

$$V_c = 1/C_c \tag{8}$$

When a cell passes through the filter it displaces a volume (V_p) of liquid given by:

$$V_p = \frac{\pi d^2 l}{4} \tag{9}$$

where d and l are the diameter and length, respectively of an individual pore. In the steady state, when a cell sweeps out a volume V_p of liquid, there must, on the average, also be swept out an equivalent volume of ($V_c - V_p$) in some number, say n, other pores. Assume that a cell (at the given driving pressure) takes an interval of time Δt to pass through a pore. Then, in order to maintain the steady state, the relations:

$$F_c \times \Delta t = V_p \tag{10}$$

$$n \times F_l \times \Delta t = V_c - V_p \tag{11}$$

must obtain. Thus for each pore containing a cell, there must on the average be n other pores containing only fluid, where:

$$n = \left(\frac{F_c}{F_l}\right)\left(\frac{V_c - V_p}{V_p}\right) \tag{12}$$

Considering a filter with N pores, the total number containing cells in the steady state ($N'f$) is then given by

$$N_f' = \frac{N}{1 + \left(\frac{F_c}{F_l}\right)\left(\frac{V_c - V_p}{V_p}\right)} \tag{13}$$

But in the steady state equation (2) becomes:

$$F_f = N_f' F_c + (N - N_f') F_i \tag{14}$$

Equations (13) and (14) may be solved for F_c and N_f', giving:

$$F_c = \frac{1}{\left(\frac{F_f}{F_i}\right) + \left(\frac{V_c}{V_p}\right)\left(1 - \frac{F_f}{F_i}\right)} \left(\frac{F_f}{N}\right) \tag{15}$$

$$N_f' = \left[\left(\frac{F_f}{F_i}\right)\left(\frac{V_p}{V_c}\right) + \left(1 - \frac{F_f}{F_i}\right)\right] N \tag{16}$$

Thus the derived quantities F_i, F_c and N_f' are expressed above in terms of F_i, F_f and V_c, which can be obtained from the measurements, as well as in terms of V_p and N, which may be estimated from the manufacturer's specifications (see below). The next step is to calculate the cellular concentration of the filtrate.

D. CELLULAR CONCENTRATION

The number of cells impinging upon the filter (equation (3)) at time t is $C_c F(t)$. These cells appear in the filtrate after an interval Δt; — *i.e.* the time required for the cells to pass through the pore. Thus at time t the number of cells being added to the filtrate is $C_c F(t - \Delta t)$. Therefore the total number of cells $v(t)$ at time t is:

$$\begin{aligned} v(t) &= \int_0^t C_c F(t - \Delta t)\, dt \\ &= C_c V(t - \Delta t) \end{aligned} \tag{17}$$

Hence the cellular concentration of all the filtrate, as a function of time, is given by:

$$C(t) = \frac{C_c V(t - \Delta t)}{V(t)} \tag{18}$$

Observe that this concentration is always less than C_c. The cellular concentration of a *sample* of filtrate collected in the interval $t_1 - t_2$ is equal to:

$$C(t_1 - t_2) = C_c \left[\frac{-V(t_1 - \Delta t) - V(t_2 - \Delta t)}{V(t_1) - V(t_2)}\right] \tag{19}$$

The volume of filtrate, which is obtained by integrating equation (5) is given by:

$$V(t) = F_f t + \frac{1}{y}(F_i - F_f)(1 - e^{-yt}) \tag{20}$$

An expression for the cellular concentration of a *sample* of filtrate, expressed as a function of time may be obtained by substituting equation (20) into (19), assuming that $t_1 > t_2 > \Delta t$:

$$C(t_1 - t_2) = \frac{C_c[F_f(t_1 - t_2) + 1/y(F_i - F_f)(e^{-yt_2} - e^{-yt_1})e^{yt}]}{F_f(t_1 - t_2) + 1/y(F_i - F_f)(e^{-yt_2} - e^{-yt_1})} \tag{21}$$

$$> C_c \text{ for all } t_1, t_2 > \Delta t$$

Thus the cellular concentration of a *sample* of the filtrate is in principle zero until time Δt, after which it rises to a value greater than C_i and then decays towards C_i (*cf.* equation 18). That this was not observed experimentally is attributed to the fact that a layer of cells adheres to the underside of the filter. A layer less than 15 microns thick would account for the discrepancy (see Fig. 3).

E. NUMERICAL CALCULATIONS

The total number of pores and the volume of individual pores may be estimated from the manufacturer's data: the filters are 150 microns thick and present a total cross-sectional area of 9.6 cm^2. Of this area 80 per cent is said to represent pores. Thus the number of pores N is calculated from the relation:

$$N = \frac{4 \times 0.8 \times 9.6}{\pi d^2} \tag{22}$$

where d is the diameter of a pore. The volume V_p of a pore is defined by equation (9). In the case of the 5 micron pores N and V_p are equal to 39.2×10^6 and 29.5×10^{-10} cc, respectively, and for the 3 micron pores to 10.9×10^6 and 10.6×10^{-10}, respectively.

In the example shown in Fig. 3 the initial flow F_i was equal to 0.83 cc/sec., the final flow F_f was 0.18 cc/sec., and the cellular concentration of the suspension was 2.92×10^6 cells/cc. Using the above figures for a 3 micron filter it is readily calculated that:

$$F_l = 7.55 \times 10^{-9} \text{ cc./sec.}$$

$$F_c = 6.44 \times 10^{-12} \text{ cc./sec.}$$

$$y = 0.022 \text{ sec.}^{-1}$$

$$V_c = 3.42 \times 10^{-7} \text{ cc.}$$

$$\Delta t = 164.5 \text{ sec.}$$

$$N_f = 0.784N$$

That the flow F_c is negligible in comparison to F_l is a typical result, observed in the calculations for every case. The velocity with which a cell moves through a pore may be calculated from Δt, and the length of the pore. In the above case this gives a value of 0.9 microns per second, which is about 1/500 of the velocity observed *in vivo*. Note that in the above example 78.4 per cent of the pores contain a cell. If the cells did not impede the flow (*cf.* equation 13) only one pore in 3,000 would be expected to contain a cell. However, the fact that less than 100 per cent of the pores contain cells supports the assumption made earlier that to a first order approximation no pore contains more than one cell.