Fluid Movement in Occluded Single Capillaries of Rabbit Omentum

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ABSTRACT

The sequence of transcapillary fluid movement during microocclusion of single capillaries was investigated on the theoretical basis of the Starling hypothesis. For most capillaries studied, the theory agrees reasonably well with the experiments with red cells used as the tags for fluid movement. By analyzing the sequence of the movement of the red cell in one single occlusion, it was possible to calculate the filtration coefficient and the effective pressure. The effective pressure ranged from 28 to 38 cm H2O. Since the arterial pressure in the capillary is about 20 to 30 cm H2O, the calculations suggest that about 10 cm H2O of the effective pressure is contributed by tissue factors, i.e., a negative hydrostatic pressure or a substantial colloidal osmotic pressure in the tissue space or both. The calculated filtration coefficient for the capillaries ranged from 0.01 to 0.07 \( \mu l/(sec \cdot mm H2O) \).

KEY WORDS

filtration coefficient effective pressure colloidal osmotic pressure Starling's hypothesis transcapillary fluid movement

In his classic analysis of fluid movement across capillary walls, Starling postulated that the capillary barrier behaves as if it were a semipermeable membrane which retains protein and that, as a consequence, the forces responsible for net shifts of fluid are the hydrostatic pressure and the colloidal osmotic pressure. Analysis of the exchange process at the level of the single capillary is complicated by the small dimensions of the capillary and the difficulty in accurately following the movement of fluid between the blood and tissue compartments. The Starling hypothesis, despite its simplicity, did not find general acceptance for several decades, until methods were developed to measure the responsible forces. Convincing evidence for the validity of this hypothesis was provided by the micro-manipulative experiments of Landis (2) and more recently by Zweifach and Intaglietta (3) on single capillaries. Support for the Starling concept for single organs is found in Papenheimer and Soto-Rivera's isogravimetric studies of isolated hind limb (4) and isovolumetric studies of the lung by Levine et al. (5).

Starling's hypothesis as applied to the fluid movement in a segment of capillary can be written as

\[ \dot{m} = K(P_c - P_t + \pi_t) \]

where \( \dot{m} \) is the rate of the net fluid movement across a unit surface area of the capillary, and \( K \) represents the filtration coefficient of the barrier. The driving forces are the hydrostatic pressure in the capillary \( P_c \) and tissue \( P_t \) and the colloidal osmotic pressure (COP) of plasma, \( \pi_p \), and of tissue \( \pi_t \).

Only scattered information exists for the capillary pressure in mammals because of the technical difficulty in making direct pressure measurements in the microcirculation system of such animals. Thus it may be more
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appropriate to designate the effective pressure \( P_e \) by

\[ P_e = P_a - P_t + \Pi, \]

(8)

which remains constant during occlusion of a single capillary.

To use the relation (Eq. 1) for calculating the filtration coefficient, it would be necessary to measure the fluid movement and the difference \( P_e - \Pi \). Under the conditions of the Landis microocclusion experiment (2) in the absence of significant forces to hinder the free movement of red cells in the occluded capillary, their relative rate of displacement should reflect the actual shift of fluid into or out of the capillary. Inasmuch as the loss of fluid from blood to the tissue is only a small fraction of the total blood flow, (approximately 1%)

\[ \Pi \]

the COP of plasma may be taken as that in sampled arterial blood. Landis measured capillary pressure directly with micro-pipets. The method, however, is traumatic and can only be done once on any one capillary. Intaglietta and Zweifach (6) developed a procedure involving an osmotic transient in which a slug of concentrated albumin solution (25%) was injected into the blood stream and the fluid movement before was compared with that after the elevation of \( \Pi \) in the same capillary. When the fluid movement and the COP before and after injection are measured, equation 1 will derive two numerical equations for \( K \) and \( P_e \), provided these parameters remain unchanged after injection. An alternative method is to change the protein concentration in the bathing solution so as to change COP in the tissue (7). All these procedures involved the data reduction of two independent occlusion procedures separated by a certain time interval. Recently, we suggested a new approach (8) to evaluate \( K \) and \( P_e \) from the data generated by a single microocclusion experiment. In the present paper, we investigate a number of implications of Starling's hypothesis for transcapillary fluid movement and report theoretical and experimental studies of the events in the capillary after occlusion.

If, at the time of occlusion, a pressure differential (Eq. 1) is present across the barrier, transcapillary fluid movement develops. This movement is small in comparison with the normal blood flow, it can be readily diffused into the large tissue space, and we may assume that the hydrostatic pressure in the tissue and the COP in the tissue remain unchanged. After occlusion, the hydrostatic pressure on the arterial side of the capillary will assume the arteriole pressure and that on the venous side the venule pressure. These pressures may not be affected by the closing of one capillary. As a whole, the effective pressure on either side of the needle is constant during the occlusion. On the other hand, the fluid movement will change the COP in the capillary until finally a stage is reached when the effective pressure is balanced by the final COP in plasma, and fluid movement no longer takes place. For example, when filtration is occurring as a result of a larger \( P_e \), the COP in plasma will gradually increase until it is balanced by the effective pressure. With the data on the initial COP, the total fluid movement, and the conservation of protein, one should be able to calculate the final COP and hence the effective pressure. Once \( P_e \) is known, analysis of the rate of fluid movement enables calculation of the averaged filtration coefficient, \( K \). The theoretical development of the implications of Starling's hypothesis for a constant \( K \) along the capillary, their experimental confirmation, and the justification of the assumptions are here presented.

Theory

In this section, we shall formulate the governing equation, present its solution, and discuss its dimensionless parameters.
FIGURE 1
Configuration of an occluded capillary. The relative distance between cell 1 and cell 2 is measured as a function of time.

Let \( x \) be the distance between two arbitrary tagged red cells as shown in Figure 1. In this system, the total volume contained between these two cells in a capillary of diameter \( D \) is \( \pi x D^2/4 \). If the capillary is a rigid tube, we have the fluid movement, \( \dot{m} \), as

\[
\dot{m} = -\frac{1}{x \pi D} \frac{d}{dt} \left( \frac{\pi x D^2}{4} \right) = -\frac{D}{4x} \frac{dx}{dt} \quad (3)
\]

Let us now evaluate the COP of plasma as a function of the distance, \( x \). The protein content of the plasma enclosed between the two red cells and the semipermeable endothelial membrane is the product of that volume of plasma and the protein concentration, \( c \), (g/100 ml of plasma), and, at any instant, it is a constant independent of the distance \( x \). Our calculations show that the COP will change in such experiments by 10 cm H₂O at most, and changes of this magnitude induce only an insignificant amount of fluid movement across the red cell membrane. Thus the volume of plasma \( V_p \) is simply the difference between the total volume of blood and the volume occupied by the red cells, whose volume \( V_r \) remains unchanged. As the latter volume is equal to the number of red cells trapped between the two tagged cells, \( N_e \) times the volume, \( V_r \), we have

\[
\frac{V_p}{(V_p)_0} = \frac{\pi x D^2/4 - NV_r}{\pi x D^2/4 - NV} = \frac{x/\pi D - H_o}{1 - H_o} = \frac{c_0}{c}, \quad (4)
\]

where \( H_o \) is the hematocrit \( (= NV_e/(\pi x D^2/4)) \) and the subscript 0 is used to designate a quantity at the time of occlusion. For the range \( 0 < c < 0.05 \) g/100 ml of plasma, the relation between the COP and the concentration can be described by the following empirical equation (9):

\[
\pi_p (\text{mm Hg}) = 0.1c + 0.16c^2 + 0.009c^3. \quad (5)
\]

Because of the third order term \( c^3 \) in the equation above, the solution is more complicated and the handling of data is difficult and time consuming. We shall, therefore, approximate the equation by the following second order formula to derive a simpler solution

\[
\pi_p (\text{mm Hg}) = 2c + 0.24c^2, \quad (6)
\]

which, in the range \( 5.5 < c < 8 \) g/100 ml encountered in occlusion experiments, differs with equation 5 by 0.3 mm Hg at most. The initial concentration \( c_0 \) can be calculated from the relation

\[
(\pi_p)_0 (\text{mm Hg}) = 2c_0 + 0.24c_0^2. \quad (7)
\]

On the basis of the above arguments and equations, the COP in plasma is given by

\[
\frac{(\pi_p)_0}{(\pi_p)_b} = \frac{1}{1 + 0.12c_0} \left( \frac{1 - H_o}{x/\pi D - H_o} \right) \left( 1 + 0.12c_0 \times \frac{1 - H_o}{x/\pi D - H_o} \right)^{1/2} \quad (8)
\]

Substituting the equation above into equation 1, we obtain the differential equation for the normalized cell distance \( X = x/\pi D \):

\[
\frac{1}{X} \frac{dX}{dT} = -\frac{(P_e) (P_e)_b}{(P_e)_b} - \frac{(\pi_p)_b}{1 + 0.12c_0} \left( \frac{1 - H_o}{X/H_o} \right) \left( 1 + 0.12c_0 \times \frac{1 - H_o}{X/H_o} \right)^{1/2} \quad (9)
\]

where the dimensionless time is \( T = 4K (P_e) d/D \). The governing equation for \( X \) can be readily solved for a prescribed effective pressure which, as discussed earlier, is not a function of \( X \).

Circulation Research, Vol. XXVIII, March 1971
Before examining the fluid movement for a periodical effective pressure $P_e$ induced by the pulsation of blood pressure, let us first consider the simpler case of a constant $P_\circ$. By definition of the subscript $\circ$, the constant is $(P_\circ)_0$. With this approximation, equation 9 can be written as

$$-\alpha T = \frac{(X-H_\circ)^2}{X} \left[ \frac{1}{(X-H_\circ)^2} - \frac{1}{1 + 0.12c_\circ} \frac{(\pi_\circ)_0}{(P_\circ)_0} \right] \frac{dX}{X} = \alpha \frac{dX}{X} + \beta \frac{dX}{X} + \gamma$$

or a direct integration with the initial condition $X = 1$ at $T = 0,$

$$X = \left( \frac{X_\circ + X_t}{1 + X_t} \right)^2 \left( \frac{X - X_\circ}{1 - X_\circ} \right)^2 = \exp\left(-\alpha T\right), \quad (10)$$

where $X_\circ$ (positive) and $-X_\circ$ (negative) are the roots of the quadratic equation

$$\frac{(X-H_\circ)^2}{X} \left[ \frac{1}{(X-H_\circ)^2} - \frac{1}{1 + 0.12c_\circ} \frac{(\pi_\circ)_0}{(P_\circ)_0} \right] = 0 \quad (11)$$

and

$$\alpha = -\frac{H_\circ^2}{X_\circ X_\circ^2}, \quad \beta = \frac{X_\circ}{X_\circ + X_\circ} \left( 1 + \frac{H_\circ}{X_\circ} \right)^2, \quad \gamma = \frac{X_\circ}{X_\circ + X_\circ} \left( 1 - \frac{H_\circ}{X_\circ} \right)^2. \quad (12)$$

As pressure pulsation is shown later to have a negligible effect on the fluid movement in a rigid permeable tube, we shall let $(P_\circ)_0$ become $P_e$, the time-averaged value of the effective pressure. It is interesting to note that a semilog plot of the function on the left hand side of equation 10, instead $X - X_\circ$ vs. $T$, yields a linear relationship with respect to $T$. As $T \to \infty$, we have $X \to X_\circ$. Since $X_\circ$ is a root of equation 11, its measurement leads to an equation for the calculation of the effective pressure

$$P_e = \frac{(\pi_\circ)_0}{1 + 0.12c_\circ} \frac{1 - H_\circ}{X_\circ - H_\circ}$$

In Figure 2, the relation between $X_\circ$ and $P_e$ for the normal blood of the rabbit ($\{\pi_\circ\}_0 = 26.5$ cm H2O and $c_\circ = 5.8$ g/100 ml) is shown for $H_\circ = 0.1$ and 0.3.

The dimensionless numbers which are likely to vary in the solution of equation 10 are $H_\circ$, $(\pi_\circ)_0$, $(\pi_\circ)_0/P_e$, and $(\pi_\circ)_0/(P_\circ)_0$ so that it would be useful to examine their influence on the movement of the cells following microocclusion. In Figure 3 the variations of $X$ for $H = 0.3$, $(\pi_\circ)_0/P_e = 0.6$, 0.8, 1.0, 1.2 and 1.4 are plotted against the dimensionless time $T$. To visualize better the dimensionless time, a physical time unit calculated with a filtration coefficient 0.02 $\mu$m/1/sec, a capillary diameter 8 $\mu$m, and an effective pressure 33 cm H2O, is associated with the abscissa in the lower part of the figure. Note that $(\pi_\circ)_0/P_e < 1$ corresponds to a fluid filtration toward the tissue, $(\pi_\circ)_0/P_e = 1$ represents the case of zero fluid movement, and $(\pi_\circ)_0/P_e > 1$ that of fluid transudation. The functional dependence of $X$ on $T$ for $(\pi_\circ)_0/P_e = 1.2$ and $H_\circ = 0.1$ and 0.3 are plotted in the upper part of Figure 4. This example illustrates fluid transudation. The case of fluid filtration with $(\pi_\circ)_0/P_e = 0.8$ is presented in the lower part of the figure. It can be seen that the slope of the curves at zero time is independent of the initial hematocrit. This point can be easily proved by...
Relations between the effective pressure and the ratio of the final distance and the initial distance between two cells. At a higher $H_o$, the fraction of plasma is smaller and the effective pressure is larger for tissue absorption ($X_o < 1$) and smaller for tissue transudation ($X_o > 1$).

When one sets $X = 1$, the resulting equation for the initial rate of the fluid movement is independent of $H_o$. When the hematocrit is higher, a smaller change in the shift of fluid is sufficient to establish equilibrium across the barrier. This feature is shown in Figure 4, which compares curves with different hematocrits.

We can now proceed from the study of a steady effective pressure to that of a sinusoidal pressure wave:

$$P_x (1 + \delta \sin \omega T), \quad (14)$$

where $\delta$ is the relative amplitude and $\omega$ the dimensionless frequency. By substituting equation 14 into equation 9, expanding it in terms of small $\delta$ and retaining the zeroth and first order terms of $\delta$, it can be estimated that

$$\Delta X \approx \delta/\omega, \quad (15)$$

where $\Delta X$ is the oscillating amplitude of the cell movement. It is seen that, at a lower frequency, the cells will tend to oscillate at a larger amplitude. For a capillary with

$$(P_x) = 26.5 \text{ cmH}_2\text{O},$$
$$c_x = 5.8 \text{ g/100 ml plasma},$$

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$D = 8.4 \mu \text{m}, K = 0.02 \mu \text{m}^2 \text{sec}^{-1} \text{cm}^2 \text{H}_2\text{O}$

$(\pi_{\text{cap}})_0 = 36.6 \text{ cm H}_2\text{O}$ and $P_s = 36.7 \text{ cm H}_2\text{O}$.

and a pulsating pressure with an amplitude 4 cm H$_2$O and a frequency $\omega = 4$ cycle/sec, the dimensionless quantities are

\[
S = \frac{4 \text{ cm H}_2\text{O}}{36.7 \text{ cm H}_2\text{O}} = 0.1 \tag{16}
\]

\[
\omega = \frac{D_s}{4KP} = \frac{3.4 \times 4 \times 2\pi}{4 \times 0.02 \times 36.7} = 72.
\]

The last equation is obtained by equating $\omega T = \pi$. Thus, from equation 15, the oscillating amplitude is

\[
\Delta X = \frac{8}{\omega} \approx \frac{0.1}{72} = 0.1 \% \tag{17}
\]

which can be neglected since the total cell movement is about 10 to 20%.

The analysis presented above is valid only when the effective pressure is independent of the position along the capillary, as in occluded vessels where fluid motion is extremely slow. For a normally flowing capillary, this assumption is violated as the hydrostatic pressure in the capillary, part of the effective pressure, is a function of the position.

Experimental Findings

The method used for the recording of red cell movement has been described in detail elsewhere (3). The essentials are as follows.

The omentum of anesthetized rabbits was viewed by a Lietz intravital microscope equipped with a long working distance condenser system. Selected capillaries were occluded with a glass microneedle controlled by a micromanipulator. As the cell movement was most rapid for the first few seconds, the events from just prior to and for 3 to 5 seconds after the microocclusion were recorded on 16-mm film using an Arriflex camera at 30 frames/sec. The film image was then enlarged in a Vanguard motion picture analyzer and

![Figure 5](http://circres.ahajournals.org/)

Cell movement at the arterial side of a capillary in the mesentery membrane. Note the difference between $P_e$ and $(\pi_{\text{cap}})_0$ is about 10 cm H$_2$O. The broken line is calculated from the theory based on the parameters given in the figure. A reasonable agreement between the theory and the experiment is obtained. The data indicate some oscillation in the cell movement at approximately that of the pulse (240 cycle/min). This may be due to the distensibility of the
measurements were made of the cell movement as a function of the time. The COP of plasma was measured by a membrane type of osmometer chamber equipped with a Statham pressure gauge of small volumetric displacement.

The recorded relative distance between two red blood cells in a capillary is presented in Figure 5. The data for the first 2 seconds were collected frame by frame and subsequently for every other frame. It is clear that the cell movement decreases as time increases. To satisfy ourselves that it indeed stops, we extended the period of microocclusion to 10 seconds. Despite the observation that the movement began to show a decreasing trend at approximately 3 or 4 seconds, the distance between two red blood cells, then began to decrease at a faster rate. This is probably due to a developing leakage in the capillary wall because of the increasing hyperosmolality of the plasma after filtration had occurred. This phenomenon was shown in five long-time occlusions. As a routine procedure in subsequent experiments, we shortened the occlusion time to about 3 seconds. The value of the final distance, \( X_\infty \), still can be extrapolated from the data.

When the experimental data were used to calculate the function specified on the right-hand side of equation 10 and were plotted on a semilog basis against time, we obtained approximately a linear relationship, as shown in Figure 6. The large deviation near the level-off stage is an artifact of the amplified logarithmic scale there. When \( X - X_\infty \) is plotted on semilog paper, a poorer fit is found with a straight line.

Three kinds of cell movements were observed. One type is represented in Figure 5. In a few instances, an apparently random cell movement was found. Obviously, a behavior of this second kind cannot be explained by a passive mechanism such as Starling's hypothesis and probably was due to incomplete occlusion of the vessel. In the third type, cell distance decreased continuously, as shown in Figure 7. Under such conditions, when the data are analyzed with equation 8, the COP at the end of recording attains values as high as 50 to 60 cm H2O. This implies that the effective pressure is of the order 50 to 60 cm Hg, which is obviously not physiological, and it would appear that either vessel leakage developed before, or is developing during, such occlusion experiments.
For the data presented here and in a large number of subsequent experiments not included in our analysis, about 7/10 of the capillary population displayed an asymptotically decreasing pattern of cell movement, 2/10 exhibited signs of leaking following mechanical obstruction and about 1/10 showed a random cell movement.

When fluid movement, \( \mu_t \), at two instances is measured, and the corresponding COP values are obtained from equation \( 8 \), then equation \( 1 \) will yield the two equations needed to calculate \( P_e \) and \( K \). However, because of the oscillation and the scattering of the data in a single occlusion run (Fig. 5), the fluid movement cannot be measured with sufficient accuracy. A numerical technique was therefore developed so as to employ all the data to find the best fit.

When we used the method of least squares to find the best fit of the experimental data by a power series, the values of \( P_e \) and \( K \) calculated did not seem to be reasonable and the match was poor. Therefore, the following numerical procedure was developed. The approximate values of the filtration coefficient and the effective pressure were first calculated from the initial and final movement of the cells. We then calculated the theoretical value of cell movement \( X_T \). The deviation of the experimental data \( X_a \) from the theoretical ones may be specified by the following standard error, \( \sigma \):

\[
\sigma = \sqrt{\frac{1}{N} \sum_{n=1}^{N} (X_n - X_a)^2}
\]

where \( n \) represents the number of the frame and \( N \) is the total number of frames used for data reduction. Then we made another numerical estimate for \( X_a \) and for \( K \) and recalculated the theoretical cell movement and the standard error. The final estimate was the one which corresponded to the smallest standard error. The smooth, solid line shown in Figure 5 is a curve so calculated. A reasonable fit with the experimental data is obtained.

When the data handling method outlined above was used, the results were presented in Table 1 for capillaries which behaved in the fashion shown in Figure 5. It was found that the data collected from every fifth frame was sufficient for the numerical calculation. The table shows less of a scatter for the effective pressure than for the filtration coefficient. The effective pressure is consistently higher than the initial COP as indicated by the observed outward movement into the tissue. It can also be seen that the capillaries on the venous side are more susceptible to leakage when mechanically stimulated.

For purposes of comparison, the filtration coefficient and effective pressure were also measured by the osmotic transient test procedure. Three sets of data are shown in Table 2. It is seen that there is only a small discrepancy between these calculated values and those presented in Table 1.

In one capillary, we made repeated measurements of fluid movement over a period of 8 hour without evidence of a leak in the vessel. The effective pressure and the filtration constant for individual runs are plotted against time in Figure 8. Note that the data vary in a minor way with the time. In all of
the other capillaries, two repeatable runs were obtained.

From Table 1, the initial fluid movement is driven by a pressure difference about 10 cm H$_2$O, and the data have a standard deviation $\sigma/(1 - X_v)$, which is the normalization of $\sigma$ with respect to the net change of fluid movement, about 10$. Thus, together with the variation in $P_e$ presented in Figure 8, it is estimated that the calculated $P_e$ may have an error about ±1 cm H$_2$O.

Discussion

From a physiological and technical point of view, the method of analysis developed in the present paper for single capillaries has a number of obvious advantages over previous procedures. First, it shortens the amount of data reduction, since only one microocclusion is needed, in contrast to the osmotic transient test for which two independent runs are needed on the same capillary at a time interval of approximately 5 to 15 minutes. The present method allows investigation of the effects of drug or other environmental change.
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Circ Res. 1971;28:358-366
doi: 10.1161/01.RES.28.3.358

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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