A Junction-Orifice-Fiber Entrance Layer Model for Capillary Permeability: Application to Frog Mesenteric Capillaries

The recent serial section electron microscopic studies by Adamson and Michel (1993) on microves s gels of frog mesentery have revealed that the large pores in the junction strand of the interendothelial cleft are widely separated 150 nm wide orifice-like breaks whose gap height 20 nm is the same as the wide part of the cleft. In this paper a modified version of the model in Weinbaum et al. (1992) is first developed in which this orifice structure is explored in combination with a random or ordered fiber matrix layer that is at the luminal surface and/or occupies a fraction of the wide part of the cleft. This basic orifice model predicts that for the measured $L_p$ to be achieved the fiber layer must be confined to a relatively narrow region at the entrance to the cleft where it serves as the primary molecular filter. The model provides a much better fit of the permeability $P$ for intermediate size solutes between 1 and 2 nm radius than the previous model in Weinbaum et al., where the junction strand breaks were treated as finite depth circular or rectangular pores, but like the previous model significantly underestimates $P$ for small ions. However, it is shown that if a small frequent pore of 1.5 nm radius with characteristic spacing comparable to the diameter of the junction proteins or a continuous narrow slit of approximately 1.5 to 2.3 nm gap height is also present in the continuous part of the junction strand, small ion permeability can also be satisfied. The 1.5 nm radius pore does not significantly change $L_p$, whereas the continuous narrow slit provides a contribution to $L_p$ that is comparable to, or in the case of the 2.3 nm slit greater than, the widely spaced 150 nm orifices. Thus, for the narrow slit the contribution to $L_p$ from the orifices can be as low as $1.0 \times 10^{-7}$ cm/s/cm H$_2$O and it is also possible to satisfy the 2.5 fold increase in permeability that occurs when the matrix is enzymatically removed from the luminal side of the cleft, Adamson (1990). The likelihood of each of these cleft structures is discussed.
Third, a cleft with a junction strand with pores and fibers throughout its wide part, while able to reconcile the data for $L_p$ and small solute permeability, could not satisfy the permeability data for solutes greater than 0.5 nm radius and could not explain the 2.5 fold increase in $L_p$ that occurs when the endothelial cell glycocalyx at the luminal front is enzymatically removed (Adamson, 1990). For the 5.5 nm radius pore and the 8 nm gap height slit, where the pores are the primary size selective filter, the addition of fibers leads to an even larger disagreement between theoretical predictions and experimental measurements. The junction pore which suggested the best agreement for $P_s$ is an infrequent 22 nm gap height break whose width is at least a standard transmission section thickness, 40 nm.

The above prediction as to the likely geometry of the large pores in the junction strand has been recently confirmed by the serial section electron microscopic studies of frog mesenters by Adamson and Michel (1993). These serial reconstructions revealed rather long breaks of typically 150 nm width and the same gap height as the wide part of the cleft. These breaks were spaced between 2140 and 4450 nm apart depending on whether conventional thin sections were used, or the breaks were identified by the leakage of the lanthanum tracer extending to the abluminal front. The junction strand pores were thus orifice like discontinuities in a narrow barrier whose depth was the diameter of the individual proteins in the strand. This type of the break is more realistically modeled by the flow through a zero thickness orifice in a channel as proposed in the appendix by Parker et al., in Adamson and Michel (1993), rather than Poiseuille flow in a rectangular channel, the pore model in Weinbaum et al. (1992). The rigorous hydrodynamic theory in Zeng and Weinbaum (1994) indicates that an orifice with the dimensions of the breaks observed in Adamson and Michel (1993) is described well by Hele-Shaw flow theory. A similar model for the junction strand breaks, to that proposed by Parker et al., is thus used in this study except that the theory is now extended to include diffusion and modified to include finite regions of fiber matrix components in the wide portions of the cleft and very small pores or slits in the continuous part of the strand to account for the permeability of small ions.

There is evidence indicating that the components of the endothelial cell glycocalyx may correspond to the fiber matrix proposed in the fiber matrix theory (Curry and Michel, 1980). Using ruthenium red staining, Luft (1966) first showed that there was a ‘fluffy’ endothelial coat. More recent studies indicate that this glycocalyx carries a negative charge and is able to bind cationized ferritin in a thin layer of 20 to 30 nm thickness near the endothelial wall (Turner et al., 1983; Adamson and Clough, 1992). There is indirect evidence based on chemical staining that matrix components may fill portions of the wide part of the cleft on the luminal side of the junction complex (Schneeberger and Hamelin, 1984). These authors showed, using an immunoperoxidase method in rat lung, that albumin bound to matrix components in the wide part of the cleft but was absent in the junctional region. More direct evidence of such components has recently been reported in the goniometric tilting of tannic acid fixed transmission electron microscopic sections of rat myocardial capillaries, Schulze and Firth (1992) and in the rapid fixation scanning electron micrographs of cultured confluent bovine arterial endothelial cells, Satcher (1993). In the former study, regularly spaced perpendicular fibers could be observed in portions of the wide part of the cleft at specific tilt angles. In the latter study fine perpendicular fibers could be clearly seen at the entrance to the cleft. In this paper we shall present results for this perpendicular fiber arrangement in either a periodic or random array.

Adamson (1990) studied the fiber matrix hypothesis by com-

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**Nomenclature**

- $a = \text{fiber radius}$
- $B = \text{half height of the cleft}$
- $B_i = B/d$
- $b = \text{half height of the junction opening}$
- $b_i = \text{half height of the continuous narrow slit in the junction strand}$
- $b_l = \text{coefficient in periodic Weierstrass expansion series}$
- $C_{i(\ell)} = \text{concentration in region } i, \ell = 1,2,3$
- $C_{L}, C_A = \text{concentration on the lumen and tissue side}$
- $C_{eff} = \text{concentration in the fiber layer in the luminal part of the cleft}$
- $D = \text{half distance between the centers of adjacent openings}$
- $D^{(\ell)}_{i(\ell)} = \text{diffusivity in region } i, \ell = 1,2,3$
- $D_{0r} = \text{free diffusivity}$
- $D_{wr} = \text{diffusivity in the restricted region } i \text{ without fiber matrix, } \ell = 1,3$
- $D_{eff} = \text{effective diffusivity when the fiber is present}$
- $d = \text{half width of the junction opening}$
- $f = \mu_{eff}/\mu$
- $f_D = D_{wr}/D_{eff}$
- $J_p = \text{volumetric flow rate per unit capillary surface area}$
- $K_p = \text{unbounded Darcy permeability cm}^2$/$\text{cm/s}$
- $K_{pe} = \text{unbounded average Darcy permeability for a random fiber matrix cm}^2$/$\text{cm/s}$
- $K_{pe,eff} = \text{bounded Darcy permeability cm}^2$/$\text{cm/s}$
- $K_{pe,eff} = \text{bounded average Darcy permeability for a random fiber matrix cm}^2$/$\text{cm/s}$
- $K = \text{permeability from the large pore system cm/s}$
- $P_s = \text{permeability from the small pore system cm/s}$
- $Q = \text{volumetric flow rate per junction opening}$
- $R_L = \text{hydraulic resistance in region } i, \ell = 1,2,3$
- $R_{Di} = \text{diffusive resistance in region } i, \ell = 1,2,3$
- $r_p = \text{radius of the circular junction pore}$
- $S_f = \text{solid fraction of the fiber matrix}$
- $S_s = \text{effective solid fraction of the fiber matrix}$
- $u, v, w = \text{velocity component in } x, y, z \text{ direction}$
- $x, y, z = \text{coordinate system}$
- $\lambda_0 = \text{eigenvalue}$
- $\lambda = d/L$
- $\Delta = \text{gap distance between the fibers}$
- $\xi, \eta = \text{coordinate system in the mapped plane}$
- $\mu = \text{fluid viscosity}$
- $\mu_{eff} = \text{effective fluid viscosity}$

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very small pores or slits, in which the junction barrier is im-
permeable except for the junctional orifices. The junctional orifices connect the two wide portions of the cleft. A fiber layer, which is either an ordered periodic or random array of perpendicular fibers, is assumed to appear at the luminal front of the cleft and also extend into the wide part of the cleft. Since a portion of this fiber layer can be part of a 20–100 nm thick surface glycoca-lyx, Turner et al. (1983) and Adamson and Clough (1992), expressions are derived for converting the unbounded matrix at the luminal surface to an equivalent thickness of bounded fiber matrix. \( L_j \) is the effective thickness of the combined equivalent fiber layer. This basic model is then modified to include a family of very small pores or slits. A continuous narrow slit of height \( b_s \) is also shown in Fig. 1(b).

For the basic orifice model only pores whose gap height \( 2b \) is equal to the gap height \( 2B \) of the wide portion of the cleft will be examined. We require that \( 2b = 2B \) since we assume that this distance is set by the background force balance between the plasma membranes when the slit width \( 2d \gg 2B \). This is consistent with observations in Adamson and Michel (1993). \( L_j \), the thickness of the junction barrier is neglected in the basic model for the orifice flow, but will be considered later in determining the resistance of the very small pores. \( L_1 \) and \( L_3 \) are the depths of the cleft on each side of the junction strand. The value of \( L_1 \) determines the position of the junctional strand. Results in a previous study (Tsay et al., 1989) indicate that the position of the junction strand has very little effect on the total hydraulic resistance across the junctional cleft; therefore, one chooses \( L_1 = L_3 \) in all our calculations. \( L \) is the total cleft depth and \( L_j \) is the depth of the fiber layer at the lumen front.

In the model, periodic and random fiber arrays are examined. For the periodic array, the fibers are represented by a periodic square array of circular cylindrical elements. These fibers are assumed to be oriented perpendicular to the plasma membranes forming the interendothelial channel as observed in the scanning electron micrographs of Satcher (1993). The fiber diameter is \( 2a \), the open spacing between fibers is \( \Delta \) and the distance between adjacent fibers is \( \Delta + 2a \).

Mathematical Modelling

A Model Geometry. A top view of our idealized model for the interendothelial cleft showing the entrance fiber layer and the junction strand is depicted in Fig. 1(a) and a three-dimensional schematic of a periodic unit is shown in Fig. 1(b). The breaks in the junction strand observed by Adamson and Michel (1993) are represented as orifice openings of dimensions \( 2d \times 2b \) in a zero thickness barrier. The spacing between orifices is \( 2D \) and these openings are periodically arranged in the plane of the strand. We shall first consider a basic model with very small pores or slits, in which the junction barrier is im-

pars normal capillary hydraulic conductivity \( L_p \) with \( L_p \) measured after partial degradation of the endothelial cell glyco-ocalyx. He observed that \( L_p \) increased from \( 2.0 \times 10^{-5} \, \text{cm/s/cm H}_2\text{O} \) to \( 4.9 \times 10^{-7} \, \text{cm/s/cm H}_2\text{O} \) after enzymatic degra-dation, a nearly 2.5 fold increase. This is the only experiment on which tries to isolate the hydraulic resistance due to fiber matrix components at the endothelial surface or in the wide part of the cleft at the luminal front. It will be shown that this behavior is not consistent with the 150 nm wide orifices being the only pathway for water and small ions, but can be explained if a significant fraction of the water and small ions passes through a narrow continuous slit in the junction strand. Evidence for such a structure has been reported in Adamson and Michel (1993).

\[ u = \frac{1 - z^2}{B^2} \]  
\[ u_0 = -\frac{B^2}{2\mu} \nabla p \]


is the velocity at the center plane $z = 0$ of the cleft. The pressure field in a Hele-Shaw flow satisfies

$$\frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} = 0$$

(3)

The boundary conditions at the boundary plasmalemma membranes of the cleft are no slip

$$u = v = 0 \text{ at } z = \pm B$$

(4)

where $u$, $v$ are the $x$, $y$ components of the fluid velocity $u$ and $w = 0$. The hydrodynamic resistance in Hele-Shaw flow originates from the shearing stress at the boundary plasmalemma membranes at $z = \pm B$ and the pressure disturbances produced by the vertical boundaries or obstacles in the $x$, $y$ plane, where no slip boundary conditions cannot be satisfied.

The effect of the hydrodynamic interaction with the crossbridging fibers in the entrance subregion of the cleft can be treated by replacing the actual fluid viscosity $\mu$ by an effective viscosity $\mu_{eff}$. The effective viscosity $\mu_{eff}$ is defined by

$$\nabla \cdot (p) = -\frac{3\mu_{eff}(U)/B^2}{\langle \rangle}$$

where $\langle \rangle$ denotes an average value over a region which is small compared to the depth of the fiber layer $L_f$. The effective viscosity can be written as $\mu_{eff}$ if $f$ is a hydrodynamic interaction function which depends on the fiber configuration, the fiber volume fraction $S_f$, and the aspect ratio $B/a$ of the fibers. It was shown in Tsay and Weinbaum (1991), where an exact infinite series solution for the square fiber array in a channel was obtained, that the following solution of the Brinkman equation gives a very good approximation for $f$ when $B/a > 5$.

$$f = \frac{\beta^2}{3(\beta - \tanh \beta)}$$

(5)

$$\beta = \frac{B}{\sqrt{K_p}}$$

$K_p$ is the Darcy permeability which describes the flow through an infinite matrix with the same fiber geometry as the interior of the bounded flow under consideration. For a two-dimensional square fiber array, $K_p$ is given by

$$K_p = 0.0572a^2 \left( \frac{\pi}{a} \right)^{2.377}$$

(6)

which is shown in Tsay and Weinbaum (1991) to be a highly accurate approximation to the exact solution for the two-dimensional periodic array in Sangani and Acrivos (1982).

If the perpendicular fibers observed in Satcher (1993) are arranged in a random array we adopt the approach described in Weinbaum et al. (1992). One retains expression (5) for $f$ but uses a Carman-Kozeny approximation for $K_p$ given by

$$K_p = \frac{(1 - S_f)^3}{S_f^2} \frac{a^2}{4C}$$

(7)

where $C$ is a fiber density correction factor. When the fibers are circular cylinders perpendicular to the flow, Happel (1959) used a periodic unit cell model with vanishing shear at the edge of the periodic unit to obtain the following approximate expression for $C$

$$C = \frac{2(1 - S_f)^3}{S_f^2} \left[ \ln \left( \frac{1}{S_f} \frac{(1 - S_f)}{(1 + S_f)} \right) \right]^{\frac{1}{5}}$$

(8)

The randomness in the distribution of fibers can now be taken into account using a stochastic model developed by Yu and Soong (1975). In this model $N$ fibers are randomly inserted into $M$ subregions and a nonuniform fiber density distribution is generated. Each subregion has a different value of $S_f$ given as

$$S_f^{(k)} = n^{(k)} \left( \frac{M}{N} \right) S_f$$

(9)

where $n^{(k)}$ is the number of fibers in subregion $i$. By assuming that there is at least one fiber in each subregion, a different value of $K_p$ is obtained for each region. The average value of $K_p$, which we denote by $K_{p,eff}$ is calculated as

$$K_{p,eff} = \sum_{n_1=1}^{N} \sum_{n_2=1}^{M} \ldots \sum_{n_M=1}^{M} K_p^{(k)} \frac{(N-M)!}{M! (n^{(k)} - 1)!}$$

(10)

The mean $K_{p,eff}$ is used to replace $K_p$ in Eq. (5) for a random fiber array.

For mathematical convenience we shall combine the unbounded fiber layer on the lumen surface observed by Turner et al. (1983) and Adamson and Clough (1992) and the cleft-spanning fibers in the wide part of the cleft and treat the entire matrix layer as if it existed in the entrance region of the wide part of the cleft. However, any thickness of surface layer can be converted into an approximate equivalent thickness of matrix inside the cleft by comparing the Darcy permeability $K_p$ (for random array) of the unbounded fiber matrix with $K_{p,eff}$ (for random array) of the bounded fiber array. In Tsay and Weinbaum (1991), the relationship between $K_p$ and $K_{p,eff}$ was developed as

$$K_{p,eff} = K_p \left( 1 + \frac{\tanh (B/\sqrt{K_p})}{B/\sqrt{K_p}} \right)$$

(11)

where $B$ is the half height of the cleft. Figure 3(a) describes the ratio of $K_{p,eff}/K_p$ as a function of the solid fraction $S_f$ of fiber matrix for a periodic and a random array. When $S_f = 0.017$ for the periodic array and $S_f = 0.085$ for the random array, both of which are determined by satisfying the measured reflection coefficient 0.9 of the frog mesentery to albumin, $K_{p,eff}/K_p$ are 0.74 and 0.89, respectively. Therefore, the resistance of a 100 nm thick fiber layer at the luminal surface is equal to that of a bounded fiber layer whose depth is 74 nm for a periodic, or 89 nm for a random array inside the cleft.

The large breaks in the junction strand are more accurately modeled as a zero thickness orifice rather than as a pore since $d >> L_z$ and $B$. Furthermore, for $D >> d$ we can neglect the interaction between adjacent breaks. The solution for a Hele-Shaw flow through a single orifice in a channel of depth $L$ is significantly simplified when the junction barrier is located in the middle of the cleft, $L_1 = L_0$, provided the effective viscosity is uniform in each region. An exact analytic solution is difficult to obtain for a finite fiber layer where $L_f < L_0$, since the pressure will not be uniform along the interface $x = L_f$ between the fiber free and fiber filled regions. A reasonable approximation, however, is to assume an average effective viscosity in region 1, $\mu^{(1)}$, which is an average of $\mu_{eff}$ in the fiber layer and $\mu$ in the fiber free subregion, which is proportional to the relative depth of each region. Thus,
conformal mapping that transforms the region, \(0 < x < L/2\), into \(0 < \xi < \infty, 0 < \eta < \infty\) in the \(\xi, \eta\) plane (Fig. 2).

The solution of (3) subject to the split boundary conditions (12), (13) and \(p = p_\|\) are unchanged. The solution for the potential pressure field in the \(\xi, \eta\) plane is given in Sneddon (1966). After transforming this solution back to the physical plane, it can be written for region 1 in Fig. 1 as

\[
p^\|_1(x,y) = \left( p_L - p_A \right) \left[ 1 - \int_0^\infty u^{-1} A(u) e^{-\theta u} \sin(\xi u) du \right] + p_A
\]

\[0 \leq x \leq \frac{L}{2}, \ y \geq 0\]

where \(p_L, p_A\) are the constant pressures at the luminal and the abluminal fronts of the cleft, \(\mu_{(i)}\), \(i = 1, 3\) are the average viscosities in regions 1 and 3, \(K\) is a complete elliptic integral of the first kind, \(P_{n-1}\) are Legendre polynomials, \(J_{2n-1}\) are Bessel functions of the first kind and the other lengths are shown in Fig. 1. This solution differs from that in Parker et al., Appendix in Adamson and Michel (1993), in that the latter solution is only valid for \(\mu_{(1)} = \mu_{(3)}\).

A similar solution to (15) can be found in region 3

\[
p^\|_3(x,y) = \frac{\mu_{(3)}}{\mu_{(1)}} \left[ p_L - p^\|_3 \left( 1 - \frac{x}{L}, y \right) \right] + p_A, \ \ \frac{L}{2} \leq x \leq L, \ y \geq 0
\]

The final expression for the flow rate through a single orifice is

\[
Q = \int_0^B \int_{-d}^d u_0(L_1, y) \left( 1 - \frac{y^2}{B^2} \right) dy dz
\]

\[
= \frac{4B^3}{3(\mu_{(1)} + \mu_{(3)})} \frac{1}{K(\alpha^2)} \left( \frac{d}{L} \right) \left( p_L - p_A \right)
\]

(17a)

where

\[
u_0(L_1, y) = \frac{\pi B^2}{2(\mu_{(1)} + \mu_{(3)})} \left[ \frac{K(1 - \alpha^2)^{-1/2}}{K(\alpha^2)} \right] \left( \frac{d}{L} \right)
\]

(17b)

The above expression for \(Q\) is easily converted into a filtration coefficient. The number of orifices per unit capillary surface area is \(L_\|/2D\), where \(L_\|\) is the total cleft length (total cell perimeter) per unit capillary surface area. Thus, the hydraulic conductivity for the large orifice system is

\[
L_{\ellen} = \frac{4B^3}{3(\mu_{(1)} + \mu_{(3)})} \frac{K(1 - \alpha^2)^{-1/2}}{K(\alpha^2)} \left( \frac{L_\|}{2D} \right)
\]

(18)

(b) Small Junctional Pore and Slit System. It is difficult to generalize the orifice solution in the previous subsection for the case where the junction barrier is permeable in the regions \(0 \leq |y| \leq D\), since \(\partial p/\partial x\) at \(x = L/2\) is unknown and varies as...
3/x

Permeability are to both agree with the measured values for
the junction strand is intact. We shall see later in the results
proximate solutions can be obtained for a continuous narrow
at the barrier. However, much simpler ap­
y
distribution to

presence for the purpose of estimating the additional contri­
leaflets of opposing membrane bilayers. Since the fractional
micrographic sections of fused junctional regions of frog mes­
which is of the order of 2 nm. This type of continuous slit is
that these small pore pathways are required in addition to the
slit or distribution of small circular pores in the region where
entrance layer for the minimum (2140 nm), maximum (4450 nm) and
average (2640 nm) spacing 2D between adjacent breaks (Adamson and
studies changes in tilt angle of a section nearly always revealed
entery capillaries in Adamson and Michel (1993). In these
suggested by the goniometric tilting of transmission electron

A narrow gap of roughly these dimensions between the outer
a frequent circular pore of this type can also account for
the cleft is filled with matrix that is a selective sieve for albumin
(7 nm diameter). We shall use this calculation to justify the
simple model for the very small circular pore that we present
next.
A second possibility for a small ion parallel pathway is a
small circular pore of radius \( r_p \) that might exist between adja­
cent proteins in the junction strand. This small pore would
have a spacing 2D = 10 nm which is approximately the diameter
of a single protein. Our results later will show that for \( r_p = 1.5 \)
nm a frequent circular pore of this type can also account for
the measured \( P \) for small ions in frog mesentery. The hydraulic
resistance of this small pore is approximately one order of
magnitude greater than the narrow slit whose relative resistance
was just estimated. This small pore, if \( L_2 = 10 \) nm, would have
a hydraulic resistance which is at least 50 times the wide part of
the cleft even if a size selective matrix for albumin was present
in the latter part of the cleft. Thus, for this small circular pore
\( R_2 \gg R_p \), \( i = 1, 3 \), and \( L_{ps} \) in (19) can be approximated by the
simple Poiseuille flow result

\[
L_{ps} = \frac{\pi r_p^4}{8 \mu L_2} \left( \frac{L_2}{2D} \right) \tag{22}
\]

The more accurate expression for \( L_{ps} \) in Tsay et al. (1989) and
Weinbaum et al. (1992), which does take account of the in­

C Diffusive Permeability.

(a) Large Breaks, Orifice Model. An equivalent theory
to that developed in subsection B(a) for filtration can be
developed for solute diffusion through the junction-orifice-
matrix geometry shown in Fig. 1. This theory closely parallels
the solution for the pressure field in the boundary value prob­
lem for determining \( L_p \) since the concentration \( C \) satisfies the
same governing Eq. (3) and boundary conditions as for \( p \). One
first needs to develop a suitable average expression for the
diffusion coefficient which is equivalent to \( \mu^I \) in Eq. (11).
The added resistance of a fiber layer at the cleft entrance
can be represented by the effective solute diffusivity \( D_{eff} \),
which accounts for both the effect of the plasmalemma membranes
and the fiber matrix. Weinbaum et al. (1992) propose the
following approximate expression for \( D_{eff} \) for solutes moving
in a confined periodic fiber array

\[
D_{eff} = D_{in} \left( 1 + \frac{r_p}{\sqrt{K_p}} + \frac{r_p^2}{3 K_p} \right)^{-1} \frac{1 - b_i S_i}{1 + b_i S_i} \tag{23}
\]

Here \( D_{in} \) is the restricted diffusivity derived in Ganatos et al.
(1981) for a sphere diffusing in a channel without matrix, but
which includes the hydraulic resistance of the plasmalemma
boundaries. The first term in parenthesis in (23) describes the
added resistance of the fiber matrix based on the motion of a
sphere in an unbounded Brinkman medium and the second
term in parenthesis describes the steric hindrance of the fiber
array. Here \( b_i \) is the coefficient of the leading term of the

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pseudo doubly periodic Weierstrass expansion series that is used to describe the disturbance produced by each fiber for the diffusion problem; \( S_0 = S_0(1 + r_s/a^2) \) represents the effective fiber solid fraction; \( r_s \) is the solute radius; \( K_p \) is the Darcy permeability for the periodic unbounded fiber array. For a random array, we replace the second term in brackets in (23) by the expression in Ogston et al. (1973) for the steric hindrance of a random matrix. Instead of (23) we have

\[
D_{\text{eff}} = D_{\text{uw}} \left( 1 + \frac{r_s}{\sqrt{K_p}} + \frac{2 r_s^2}{3 K_p} \right) \exp \left[ -S_f \frac{2 r_s}{a} \right]
\]  

(24)

The randomness in the distribution of fibers is taken into account using the same stochastic model described previously for \( K_p \) in Eqs. (7) and (10).

In expressions (23) and (24) the value of the diffusion coefficient in the surface glycocalyx differs from that in the bounded fiber layer inside the cleft that \( D_{\text{uw}} \) is replaced by \( D_{\text{uw}} \), the free diffusion coefficient without matrix and restricted boundaries. The variation of \( D_{\text{eff}} \) with solute radius for the same periodic or random matrix for which \( K_{p,\text{eff}} \) was calculated in Fig. 3(a) is shown in Fig. 3(b) for both the surface layer and the bounded cleft entrance layer.

The equivalent expression to Eq. (11) for \( \mu^{(A)} \) which takes account of the additional diffusional resistance of the fiber layer is

\[
D^{(A)} = \frac{D_{\text{uw}}}{D_{\text{uw}}L_f + (L_f - L_f)L_{\text{eff}}} (25)
\]

and a similar expression can be written for region 3. The final expression for \( P \) which corresponds to the solution for \( L_{ef} \) in Eq. (18) is

\[
P = \frac{4BD^{(1)}D^{(3)}}{D^{(2)} + D^{(3)} - D^{(2)}/(1-\alpha)} \frac{K(1-\alpha^2)^{1/2}}{D^{(2)}} \frac{L_{\text{eff}}}{2D}
\]

(26)

where \( \alpha, K, L_f, D \) have the same definitions as in (18).

(b) Small Junctional Pore and Slit System. The simplified one-dimensional model for the additional diffusive permeability \( P \) for the narrow slit closely parallels that already described in determining \( L_{\text{eff}} \) for the narrow slit. Instead of Eq. (19) one has

\[
P = (P_{D_1} + P_{D_2} + P_{D_3})^{-1}
\]

(27)

where the \( R_{D_i} \) are the diffusive resistances in each region. For the narrow slit these are given by

\[
R_{D_i} = \frac{1}{2BD^{(i)}D_{\text{uw}}} [L_{\text{f}_i} + (J_{F_i} - 1) L_{\text{f}_i}] \quad i = 1, 3
\]

(28a)

\[
R_{D_2} = \frac{L_2}{2BD_2D^{(3)}},
\]

(28b)

where \( f_d \) is equivalent to \( f \) in Eq. (20a) and is given by \( D_{\text{uw}}/D_{\text{uw}} \). For small ions \( f_d = 1 \), and \( R_2/R_3 = 0.5 \) if \( B/D_2 = 10 \) and \( L_2/L_3 = 20 \). Thus, in contrast to the filtration problem for the narrow slit, the diffusive resistance of the narrow slit is significantly less than the width of the cleft.

One can similarly show that for very small circular pores of radius \( r_p \), \( R_2/R_3 = (4BD \pi r_p^2)/(L_2/L_3) \), which is of order (1) for the same values of these geometric parameters cited earlier for filtration. This is a maximum estimate for this ratio which neglects the additional resistance of the fiber matrix in the wide part. The diffusive resistance of the wide part of the cleft can, therefore, not be neglected in calculating \( P \) for small circular pores and one cannot used a simple result equivalent to Eq. (22) for \( L_{\text{uw}} \) for determining \( P \).

The appropriate diffusion problem for a junction strand barrier with small circular pores of radius \( r_p \) and spacing \( 2D \) separating regions where there may be a fiber matrix layer of thickness \( L_{\text{uw}} \) at the luminal front is defined by the boundary conditions:

\[
\frac{\partial C_1}{\partial y} = 0 \quad y = 0, \quad D
\]

(29a)

\[
\frac{\partial C_1}{\partial x} = 0 \quad d \leq |y| \leq D, \quad x = L_1
\]

(29b)

\[
\frac{\partial C_1}{\partial x} = Q \quad |y| < d, \quad x = L_1
\]

(29c)

in the fiber free portion of region 1. Here \( Q \) is the diffusive flux through the small pores in region 2 and \( d \) is an equivalent entrance width in the wide part which is determined by requiring that \( 4bd = \pi r_p^2 \). At the interface with the fiber layer we require

\[
C_1 = C_{1f}, \quad x = L_f
\]

(29d)

\[
f_d \frac{\partial C_1}{\partial x} = Q \quad x = L_f
\]

(29e)

Equivalent boundary conditions can be written for region 3. The expression for \( Q \) is written in terms of a one-dimensional diffusion equation in which the continuity of concentration and flux at the pore entrance and exit are satisfied. At \( x = 0 \), \( C_{1f} = C_i \) and at \( x = L, C_3 = C_A \).

The solution to the boundary value problem just summarized leads to the following expressions for \( R_{D_1} \) and \( R_{D_2} \) in (27)

\[
R_{D_1} = \frac{1}{2BD_{1f}D_{\text{uw}}} \left[ L_{\text{f}_1} + (J_{F_1} - 1) L_{\text{f}_1} - \frac{2}{\alpha} \sum_{i=1}^{3} F_{n,i} \sin^2(\theta_{n,i}) \right]
\]

(30a)

\[
F_{n,i} = -\frac{f_d + \coth(\theta_{n,i}) tanh(\theta_{n,i}L_{\text{f}_i})}{f_d tanh(\theta_{n,i}L_{\text{f}_i})} \quad i = 1, 3,
\]

\[
\theta_{n,i} = \frac{n \pi}{D}, \quad n = 1, 2, 3, \ldots
\]

In region 2, the small circular pore, the resistance is

\[
R_{D_2} = \frac{L_2}{2D} \frac{1}{\pi r_p^2}, \quad I_{D_2} \quad (30b)
\]

where \( D^{(2)} \) is the restricted diffusion coefficient in the junction pore region. An approximate solution for \( D^{(2)} \) for circular pores is given in Curry (1984).

Model Parameters

We shall first identify all the parameters that appear in the model and estimate, where available, the anticipated range of values. The values for the cleft height, \( L = 400 \) nm, and total cleft length per unit area, \( L_3 = 2000 \) cm/cm², are based on the measurements for single perfused vessels in frog mesentery by Clough and Michel (1988). We believe the most reliable measurement of the gap height \( 2B \) of the wide part of the cleft is 20 nm, Frokjaer-Jensen (1991). These average values of \( L, L_2 \) and \( 2B \) are close to the mean values reported by other investigators for frog mesentery. The recent study by Adamson and Michel (1993) has provided the first reliable bounds on the size and spacing of the large breaks in the junction strand of frog mesentery capillaries. Two estimates have been obtained. Conventional serial construction has revealed 3 breaks in a distance of 13.36 \( \mu \)m or a spacing of 4450 nm. Lanthanum labeling of consecutive sections has revealed eleven breaks where the tracer has penetrated to the abluminal side over a distance of 23.56 \( \mu \)m. The smaller average spacing of these breaks, 2140 nm, might be due to the presence of shorter breaks that are not clearly detectable in a single transmission section. This possibility is supported by a theoretical model for the convective diffusive wake of tracers on the downstream side of the junction strand, Fu et al. (1995). The latter study predicts that due to lateral spreading, the width of the lanthanum wake from a short break can be several times the length of the break. Thus, the wake may be seen whereas the break itself could be missed.
The average spacing for all 14 breaks is 2640 nm while their average width $2d = 150$ nm. Results will be presented for all three orifice spacings, $2D = 2140$, 2640 and 4450 nm. Since $2d = 150$ nm is much greater than the thickness of the junction strand $L_2 = 11$ nm and the cleft height $2B = 20$ nm, we shall treat the junction strand as a zero thickness barrier for the case of the large orifice. For the small pore or narrow slit the thickness $L_2$ will be considered.

The largest uncertainties in the model are the measured value of $L_p$ and the structure of the fiber matrix. The measured value of $L_p$ in Adamson (1990) is $2.0 \times 10^{-7}$ cm/s/cm H$_2$O. This is significantly less than the measured average value $L_p = 5.9 \times 10^{-7}$ cm/s/cm H$_2$O in Clough and Michel (1988) and $4.0 \times 10^{-7}$ cm/s/cm H$_2$O in Adamson and Michel (1993) for frog mesentery. For reasons discussed in Adamson and Michel (1993) all three measurements could be overestimated by a factor which could be as large as 1.8. A major cause of this overestimate is that centerline rather than average values of the red cell velocity were used to calculate $L_p$. We shall present results for $L_p$ which lie in the range $1.4 \times 10^{-7}$ cm/s/cm H$_2$O and $2.0 \times 10^{-7}$ cm/s/cm H$_2$O as the most likely reference value.

As discussed in the introduction there are no definitive studies on the fiber structure of the matrix. In the fiber matrix theory it is generally assumed that the dominant matrix structures determining resistance and the size of the molecular sieve are the GAG side chains of the proteoglycans. It is generally accepted that albumin, 7.0 nm diameter, does not easily pass through this matrix and has a reflection coefficient $\sigma$ that is close to 0.9, Michel (1988). The sialic acid side chains in the GAG have a radius $\sigma = 0.6$ nm. If these are ordered in a regular array by albumin, as proposed in Michel (1988), then $\Delta = 7$ nm for the perpendicular array shown in Fig. 1. For these values of $\sigma$ and $\Delta$, the fiber volume fraction $S_f$ is 0.017. For the random fiber array we require that the value of $S_f$ satisfy the measured value for $P$ for albumin. This corresponds for a random matrix to $S_f = 0.085$ and a reflection coefficient $\sigma$ of 0.95 (Curry, 1984). The principal unknown for either matrix is the effective depth of the fiber layer $L_f$. This effective depth is determined by requiring the total hydraulic resistance of the surface layer plus the entrance layer in the cleft satisfy the measured values of $L_p$. As discussed earlier and shown in Fig. 3(a) any thickness of surface fiber layer can be converted to an equivalent thickness of bounded cleft entrance layer in determining $L_f$.

Results

(a) Hydraulic Resistance; Fiber Layer Depth. We shall first present results for the change in $L_p$ with fiber layer thickness $L_f$ for the basic orifice model and later in subsection (d) show how this will be altered if very small pores or a narrow slit are present in the continuous region of the junction strand. The filtration coefficient has been calculated for each of the three orifice spacings obtained from the Adamson and Michel (1993) data in Figs. 4(a), (b) for the periodic and random perpendicular fiber arrays in that order. The decrease in $L_p$ as the fractional depth $L_f/L$ of the cleft is increased is shown in this figure. One observes for the periodic perpendicular array in Fig. 4(a) that the measured value, $L_p = 2.0 \times 10^{-7}$ cm/s/cm H$_2$O, is achieved for $2D/L = 0.26$ or $L_p = 104$ nm. At the other extreme, $2D = 4450$ nm, one is able to satisfy the measured value of $L_p$ for the periodic fiber array when $L_f = 12$ nm. The corresponding values for the fiber layer depth for the random perpendicular array are $L_f = 23$ nm for $2D = 2140$ nm and $L_f = 3$ nm for $2D = 4450$ nm.

The first important observation from the above calculations is that the reference value of $L_p = 2.0 \times 10^{-7}$ cm/s/cm H$_2$O cannot be achieved, using the basic orifice model, unless the matrix occupies a relatively small fraction of cleft depth. Since this value of $L_p$ is at the lower end of the measured values for the hydraulic conductivity, the calculated depths of the fiber layer cited above are close to maximum bounds if the only pathway for water is the 150 nm orifice observed in Adamson and Michel (1993). Considering that part of the fiber matrix is unbounded on the luminal surface, the total depth of the effective fiber layer could be up to 25 percent greater than the estimates given above for the periodic fiber array if $S_f = 0.017$, see Fig. 3(a). The predicted depth of the periodic fiber layer is significantly larger for the same $L_p$ than the random layer. The experiments of Adamson and Clough (1992) indicate that the surface glyocalyx by itself is 60–100 nm thick in a plasma perfusate and thus only the ordered periodic structure would be compatible with this observation, see discussion. The second important observation is the increase in $L_p$ that results when the fiber layer is enzymatically removed as described in the experiments of Adamson (1990). In interpreting the results of this experiment one assumes that the enzymatic degradation has not also altered the structure of the junction strand. If the junction strand is unchanged, one can attribute the entire increase in $L_p$ to the enzymatic removal of the matrix. This is described in the model by setting $L_f/L = 0$. In this limit Adamson found that $L_p$ increased to $4.9 \times 10^{-7}$ cm/s/cm H$_2$O from a control value of $2.0 \times 10^{-7}$ cm/s/cm H$_2$O. One concludes from Figs 4(a), (b) that this 2.5 fold increase in $L_p$ can only be achieved when the orifice spacing $2D = 2140$ nm if the entire transendothelial water flux is accounted for by the large orifice-like breaks.

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(b) Permeability Intermediate Size Solutes. The solutions for the solute permeability $P$ corresponding to the calculations for $L_p$ in Figs. 4(a),(b) for the basic orifice model are shown in Figs. 5(a),(b). All the curves in Fig. 5 satisfy the measured $L_p$ of $2.0 \times 10^{-7}$ cm/s/cm H$_2$O. One observes that the best fit for the intermediate size solutes between 1 and 2 nm radius is obtained for the most distant orifice spacing, $2D = 4450$ nm, for both the periodic and random fiber layers. For this value of $2D$ the fiber layer at the luminal surface is very thin (12 or 3 nm) and the enzymatic degradation of the fiber layer would lead to only a small increase in $L_p$. The solution for $2D = 2140$ nm, which did account for the 2.5 fold increase in $L_p$ discussed in subsection (a) is thus not consistent with the curves which provide the best fit for $P$ for intermediate size solutes. All the curves in Figs. 5(a),(b) significantly underestimate $P$ for small ions. We shall show in subsection (d) that both these difficulties can be accounted for if a second pore system for water and small solutes is included.

(e) Varying $L_p$ of the Large Pore System. All the curves in Fig. 5 satisfy $L_p = 2.0 \times 10^{-7}$ cm/s/cm H$_2$O. However, as noted in the section describing choice of parameters, typical values of $L_p$ lie in the range $2-4 \times 10^{-7}$ cm/s/cm H$_2$O. In addition, if a second pore system does exist in parallel with the large orifice-like breaks, $L_p$ for the orifice pathway (large pore system) by itself could be less than $2.0 \times 10^{-7}$ cm/s/cm H$_2$O. The latter possibility might allow for a thicker fiber layer which fills the luminal side of the wide part of the cleft. Both these possibilities are examined in Figs. 6(a),(b), where $L_p$ is allowed to vary between 1 and $4 \times 10^{-7}$ cm/s/cm H$_2$O. In Fig. 6(a) a thin fiber layer ($L_p = 20$ nm) is maintained at the luminal surface and $L_p$ is varied by changing the orifice spacing. One observes that the solution for $P$ in which $L_p = 1.0 \times 10^{-7}$ cm/s/cm H$_2$O provides a remarkably good fit for the intermediate size solutes which is superior to the curves shown in Fig. 5(a). In general the smaller the value of $L_p$ associated with the large pore system the better the fit for the intermediate size solutes. The difficulty with this large $L_p$ is that both these difficulties could be observed for if a second pore system for water and small solutes is included.

In Fig. 6(b) we have repeated the calculation in Fig. 6(a), but this time for a thicker fiber layer that fills the entire wide part of the cleft on the luminal side of the junction strand. The interesting observation is that we again obtain a best fit for the intermediate size solutes when $L_p = 1.0 \times 10^{-7}$ cm/s/cm H$_2$O, but this time the orifice spacing is only 2700 nm and thus lies within the range of spacings, 2140 to 4450 nm, observed in the Adamson and Michel's experiments and very close to their mean value 2640 nm. One also observes that if $L_p$ of the large pore orifice pathway is $2.0 \times 10^{-7}$ cm/s/cm H$_2$O or greater the orifice spacing will be significantly less than that observed in Adamson and Michel (1993) if $L_p = L_d$. The primary difficulty with the $L_p = 1.0 \times 10^{-7}$ cm/s/cm H$_2$O solution in Fig. 6(b) is that it underestimates by an order of magnitude $P$ for small ions. This feature and the fact that $L_p$ for the larger pore system is only a half or less of the total $L_p$ suggest that a second small pore parallel pathway is needed. The solutions for the two highest values of $L_p$ are only approximate since the spacing $2D$ is not large enough to ignore orifice-orifice interaction.

(d) Permeability to Small Ions, Small Pore Pathway. As discussed in the theoretical formulation two alternate small pore pathways have been examined. The first is a small circular pore that might exist between adjacent proteins in an otherwise continuous junction strand and the other is a narrow continuous slit that runs along the entire length of the strand. The latter is suggested by the goniometric tilting experiments in Adamson and Michel (1993). In nearly all regions where the outer membrane leaflets appeared to be fused a very thin slit

![Graph showing permeability variation](image)

Fig. 6(a) Effect of changing $L_p$ on permeability $P$ for fixed fiber layer thickness $L_d$. Spacing $2D$ determined by requiring $L_p$ to satisfy specified values. In (a) there is thin fiber entrance layer $L_d = 20$ nm, whereas in (b) matrix fills entire luminal side of cleft $L_d = L_0$. All lengths are in nm and units of $L_p = 10^{-7}$ cm/s/cm H$_2$O. $S_0 = 0.017$ for periodic matrix, $L_0 = L_d$. $L_0 = 0$.

(i) Small Circular Pore. Several different size small circular pores were examined in parallel with the basic large pore orifice pathway. The solution that provided the best overall fit for $P$ for both small ions and intermediate size solutes was a value for $r_p$ of approximately 1.5 nm. This solution is shown in Fig. 7(a). The dotted curve is the contribution to $P$ of just this small circular pore, the dashed curve is the large orifice solution by itself ($L_d = 0$) and the solid curve is the superposition of the two contributions. The dash-dot curve is the combined solution for the maximum orifice spacing $2D = 4450$ nm in Adamson and Michel (1993). The solution for $P$ for the latter spacing provides an even better fit to the experimental data for $P$ than the solution for $2D = 2640$ nm. For both cases the filtration coefficient for the large orifice system, $L_{fit}$, is $1.7 \times 10^{-7}$ cm/s/cm H$_2$O. This can be achieved when $L_p = 95$ nm for $2D = 2640$ nm and $L_p = 26$ nm for $2D = 4450$ nm. One observes that this type of small pore contributes only insignificantly to filtration, $L_{fit} = 0.3 \times 10^{-7}$ cm/s/cm H$_2$O, since its hydraulic resistance is very large, but greatly enhances small ion permeability. The contribution of this pathway falls off very rapidly with increasing solute size and is negligible for
solutes larger than 1 nm radius. For the $2D = 2640 \text{ nm}$ and $L_p = 95 \text{ nm}$ solution the fiber layer constitutes 52 percent of the total resistance, whereas for the $2D = 4450 \text{ nm}$ and $L_p = 26 \text{ nm}$ solution, the fiber layer constitutes less than 25 percent of the total cleft resistance. Thus, only the combined small and large pore model for $2D = 2640 \text{ nm}$ is also able to explain Adamson’s (1990) experiment in which $L_p$ was increased 2.5 fold by the enzymatic degradation of the fiber layer.

The principal problem associated with this combined small and large pore model is that it does not account for the variation in $L_p$ and $P$ that has been observed between individual vessels in the frog mesentery. In general, it has been observed that the variation in $L_p$ roughly parallels changes in small solute permeability. This suggests that a significant fraction of the water and small solutes enter through the same pathway, (Curry, 1979; Michel, 1984). This type of small pore is not consistent with this observation since the contribution to $L_p$ is much smaller than the contribution to $P$ for small ions.

(ii) Narrow Slit. The results for the narrow slit pathway are shown in Fig. 7(b) for $2D = 2640 \text{ nm}$. Numerical tests revealed that a nearly optimal fit for solutes less than 1 nm radius could be achieved with a slit height $2b_s = 1.5 \text{ nm}$. Results for two different slit depths $L_2$ are shown, 5.5 and 11 nm. A principal difference between the small circular pore solutions in Fig. 7(a) and the slit solutions in Fig. 7(b) is that the contribution of $L_p$ to the total $L_p$ is substantially larger for the narrow slit geometry. When $L_2 = 11 \text{ nm}$ the contribution to $L_p$ is one-third of the large pore orifice pathway and $L_p$ is reduced to $1.5 \times 10^{-7} \text{ cm/s/cm H}_2\text{O}$. This smaller $L_p$ allows for a thicker fiber layer while still maintaining the orifice spacing at $2D = 2640 \text{ nm}$. This thicker fiber layer of depth 118 nm contributes 55 percent of the total hydraulic resistance. If this fiber layer were enzymatically removed $L_p$ would increase to $4.4 \times 10^{-7} \text{ cm/s/cm H}_2\text{O}$. This solution thus closely predicts the 2.5 fold increase in hydraulic permeability that was observed in Adamson (1990) after enzymatic degradation of the surface glyocalyx. The solution for $L_2 = 5.5 \text{ nm}$ also provides a slightly better agreement for intermediate size solutes between 1 and 2 nm radius than the $L_2 = 11 \text{ nm}$ solution but this solution requires that the matrix fill half the cleft depth, $L_f = L_1$.

In Fig. 7(c) we have plotted a second set of solutions for the narrow slit geometry with slit height $2b_s = 2.3 \text{ nm}$ which was the measured value of the narrow transmucosal region between outer membrane leaflets in Adamson and Michel (1993). In this case the contribution to $L_p$ from the narrow slit $L_p = 1.4 \times 10^{-7} \text{ cm/s/cm H}_2\text{O}$ is more than twice the contribution to $L_p$ from the large breaks, which is only $3.6 \times 10^{-7} \text{ cm/s/cm H}_2\text{O}$. Besides, in order to satisfy the total $L_p = 2.0 \times 10^{-7} \text{ cm/s/cm H}_2\text{O}$, the fiber matrix needs to fill entire depth of the cleft and thus provides 64 percent of the total resistance. If the fiber matrix was enzymatically removed from the entire wide part of the cleft, $L_p$ would increase to $5.5 \times 10^{-7} \text{ cm/s/cm H}_2\text{O}$ and slightly overestimate the 2.5 fold increase in $L_p$ observed in Adamson (1990). Such complete removal may not be possible if the proteolytic enzymes cannot penetrate the junctional region. This narrow slit geometry somewhat overestimates the permeability of small ions of radius $< 1 \text{ nm}$; however, this combined narrow slit and orifice pore system provides the best agreement of all the solutions for intermediate size solutes $> 1.5 \text{ nm}$ radius. This solution also has the advantage that changes in $L_p$ will roughly parallel changes in small ion permeability.

Discussion

The recent experiments of Adamson and Michel (1993) have provided valuable new data on junction strand structure in frog mesentery which have made it feasible to quantitatively evaluate the permeability pathways in the interendothelial cleft in a manner that, heretofore, had not been possible. By providing bounds on the size and frequency of the large breaks in the junction strand these experimental studies have made it
possible to realistically assess the contributions to permeability of other structural components such as the thickness of the fiber layer and other alternate pathways for small ion permeability which the large pore system cannot account for.

The solutions for the basic orifice model without the narrow slit in Fig. 4 clearly indicate that neither an ordered or random fiber matrix with the proper reflection coefficient for albumin can exist throughout the width of the cleft. This matrix would reduce \( P_r \) to significantly less than 1.0 \( \times 10^{-7} \) cm/s/cm H\(_2\)O for the observed frequency of orifice-like breaks, 2140<2D<4450 nm, observed in Adamson and Michel's experiments. However, within this range of 2D, it is possible to have an ordered matrix with the appropriate selectivity for albumin, which fills the entire luminal side of the wide part of the cleft if \( P_r \) of the orifice pathway were reduced to 1.0 \( \times 10^{-7} \) cm/s/cm H\(_2\)O, see solid curve in Fig. 6(b). According to Fig. 4(a) these fibers would contribute approximately five times the resistance of all remaining structures in the cleft and thus \( P_r \) would increase five-fold if this layer were removed by enzymatic degradation. This increase is too large and the present calculations suggest that the thickness of the ordered part of the fiber layer is more likely of the order of 100 nm in frog mesentery, see Fig. 4(a), since this value for \( P_r \) provides the best agreement with Adamson's (1990) 2.5 fold measured increase in permeability after enzymatic degradation. Matrix could be present in other regions but it would have to be much more diffuse and not ordered. Such a scenario is consistent with a model in which the albumin orders the fibers in the entrance region of the cleft and at the luminal surface and this forms a selective network which makes it difficult for albumin to penetrate further. The experiments of Schneeberger and Hamlin (1984) support this picture since staining for albumin was observed in the wide part of the entrance region of the cleft but absent from deeper regions near the junctional complex.

The foregoing estimate of \( P_r \) is consistent with the recent studies of Adamson and Clough (1992) on the thickness of endothelial cell glycocalyx in frog mesentery capillaries. These investigators used cationized ferritin as a marker of cell surface glycocalyx and observed that when the vessels were fixed after perfusion with frog plasma, the ferritin penetrated the outer region of the glycocalyx to a depth of 20 to 30 nm and that there was a separation layer of 32 nm thickness between this layer and the membrane surface. The total thickness of the glycocalyx, \( \sim 60 \) nm, (equivalent \( L_r \) of 42 nm, see Fig. 3(a) for \( S_f = 0.017 \) for periodic array) is thus about 40 percent of the 100 nm thickness that we have estimated is necessary to predict the 2.5 fold increase in \( P_r \) after enzymatic degradation. The thickness of ferritin (11 nm) was determined by enzymatic digestion, and this value would increase fivefold if this layer were the cleft and thus \( P_r \) would increase fivefold if this layer were removed by enzymatic degradation. It is clear that the orifice-like breaks in the junction strand cannot account for small ion diffusive permeability, even if they are spaced near the low end, 2D = 2140 nm, of Adamson and Michel's measurements, see Figs. 5(a),(b). This provides strong evidence for a second family of small pores. Two alternate hypotheses for this small pore family have been advanced in the present paper, a small circular pore of 1.5 nm radius and a narrow continuous slit of 1.5 to 2.3 nm height. Both pores can account for the permeability of solutes of less than one nm radius, but only the narrow slit can significantly contribute to filtration, see Figs. 7(a),(b),(c). When the filtration coefficient of the narrow slit pathway \( P_m \) is 1.0 \( \times 10^{-7} \) cm/s/cm H\(_2\)O or larger, the spacing of the orifice breaks can be sufficiently far apart to both satisfy Adamson and Michel's measurements of 2D and provide very good agreement with the measurements of \( P \) for intermediate size solutes. The predicted thicknesses of the fiber layer for the solutions in Fig. 7(b) are also consistent with the 2.5 fold increase in hydraulic conductivity that would result from enzymatic degradation of the matrix of the luminal side of the junction strand.

An intriguing feature of the solute permeability curve is the break in slope for solutes less than or greater than approximately one nm radius. The proposed two pore, larger orifice-narrow slit, model provides a rational explanation for this behavior that appears to be quantitatively consistent with the large body of data on selectivity, small ion permeability, hydraulic conductivity and permeability of intermediate size solutes for frog mesentery capillaries. The 2.3 nm slit provides the best prediction for \( P \) for solutes of radius > 1 nm, but the break in the curve for \( P \), is shifted too far to the right, see Fig. 7(c), whereas the 1.5 nm slit correctly predicts the break but somewhat overestimates \( P \) for \( r_s > 1 \) nm.

An area that requires further examination is the role of the depth of the narrow slit. This depth reflects the number of continuous junction strands that appear in series. A further observation in frog mesentery capillaries is that small ion permeability and hydraulic conductivity appeared to be roughly coupled in different microvessels in Curry (1979) and Michel (1984), and increase or decrease in parallel with one another. Figure 7(b) suggests that this increase or decrease may be associated with the average number of continuous junction strands that the water and small ions cross in traversing the cleft. The small circular pore in an otherwise continuous strand would not qualitatively give this behavior since its contribution to \( P_r \) is too small. The narrow slit model, however, can account for a significant water flux and thus yield results that are roughly consistent with this coupled behavior. The depth of a single slit \( L_p \) has been estimated in this study, \( L_p = 4450 \) nm. This is a crude guess since the actual structure of the narrow slit region is well beyond the resolution of the electron micrographs of the narrow slit observed in Adamson and Michel (1993).

A fascinating area of new research which should help elucidate the two families of pores proposed in this study is the analysis of the time dependent wakes that should be produced by a small labeled tracer passing through the cleft. Existing studies of labeled tracer have ignored the temporal aspects of tracer labeling and the importance of the time dependent filling of the subendothelial space in the vicinity of each pore pathway. While the present theory predicts that the flux of small ions is primarily attributed to the narrow slit, small pore pathway, the flux per unit junction length is still significantly larger in the vicinity of an orifice break. The local subendothelial space would, therefore, fill more rapidly in the vicinity of an orifice break and approach steady state values more rapidly. The concentration along the entire cleft depth would increase in such a region and the cleft would appear to be labeled all the way to the abluminal side. In contrast, in the narrow slit region the subendothelial space would fill much more slowly and the labeling of the cleft would appear to change with time as the concentration at the cleft exit gradually rose.
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American Physiological Society, Bethesda, MD.
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APPENDIX
The dimensionless pressure and the length can be defined as
\[ \bar{p}(x, y) = \frac{p(x,y) - p_A}{p_l - p_A} = \frac{x}{L} = \frac{y}{L} \] (1)
The solution for a Hele-Shaw flow through a single orifice in a channel of depth \( L \) is significantly simplified when the junction barrier is located in the middle of the cleft because of the linearity of a Hele-Shaw flow, Eq. (3) in the paper and the symmetric geometry about the junction barrier \( x = 1/2 \). Therefore the potential pressure distribution in region 3 has the following relationship with that in region 1
\[ \bar{p}^{(3)}(x, y) = C_1[1 - \bar{p}^{(1)}(1 - x, y)] \frac{1}{2} \leq x \leq 1, \quad \bar{y} \geq 0 \] (2)
At the orifice, the pressure and the velocity should be continuous
\[ \bar{p}^{(1)}(x, y) = \bar{p}^{(3)}(x, y) \] (3)
Substituting (2) into (4), the constant \( C_1 \) is obtained as
\[ C_1 = \frac{\bar{p}^{(3)}}{\mu^{(0)}} \] (6)
One can also show from (2) and (5) that
\[ \frac{\partial \bar{p}^{(1)}}{\partial \bar{y}} = \frac{\partial \bar{p}^{(3)}}{\partial \bar{y}} = 0 \quad \bar{x} = \frac{1}{2}, \quad 0 \leq \bar{y} \leq \frac{d}{L} \] (7)
Equation (7) indicates that the pressure is uniform along the orifice and the value at the orifice can be obtained from (2), (3) and (6)
\[ \bar{p}^{(2)}(x, y) = \frac{\bar{p}^{(3)}(x) + \mu^{(0)}p_A}{\mu^{(1)} + \mu^{(0)}} = \frac{1}{2}, \quad 0 \leq \bar{y} \leq \frac{d}{L} \] (8)