A 1-D model to explore the effects of tissue loading and tissue concentration gradients in the revised Starling principle

Xiaobing Zhang,1 Roger H. Adamson,2 Fitz-Roy E. Curry,2 and Sheldon Weinbaum1

1Departments of Biomedical and Mechanical Engineering, The City College of the City University of New York, New York, New York; and 2Department of Physiology and Membrane Biology, University of California, Davis, California

Submitted 2 November 2005; accepted in final form 24 July 2006

Zhang, Xiaobing, Roger H. Adamson, Fitz-Roy E. Curry, and Sheldon Weinbaum. A 1-D model to explore the effects of tissue loading and tissue concentration gradients in the revised Starling principle. Am J Physiol Heart Circ Physiol 291: H2950–H2964, 2006. First published August 11, 2006; doi:10.1152/ajpheart.01160.2005.—The recent experiments in Hu et al. (Am J Physiol Heart Circ Physiol 279: H1724–H1736, 2000) and Adamson et al. (J Physiol 557: 889–907, 2004) in frog and rat mesentery microvessels have provided strong evidence supporting the Michel-Weinbaum hypothesis for a revised asymmetric Starling principle in which the Starling force balance is applied locally across the endothelial glycocalyx layer rather than between lumen and tissue. These experiments were interpreted by a three-dimensional (3-D) mathematical model (Hu et al.; Microvasc Res 58: 281–304, 1999) to describe the coupled water and albumin fluxes in the glycocalyx layer, the cleft with its tight junction strand, and the surrounding tissue. This numerical 3-D model converges if the tissue is at uniform concentration or has significant tissue gradients due to tissue loading. However, for most physiological conditions, tissue gradients are two to three orders of magnitude smaller than the albumin gradients in the cleft, and the numerical model does not converge. A simpler multilayer one-dimensional (1-D) analytical model has been developed to describe these conditions. This model is used to extend Michel and Phillips’s original 1-D analysis of the matrix layer (J Physiol 388: 421–435, 1987) to include a cleft with a tight junction strand, to explain the observation of Levick (Exp Physiol 76: 825–857, 1991) that most tissues have an equilibrium tissue concentration that is close to 0.4 lumen concentration, and to explore the role of vesicular transport in achieving this equilibrium. The model predicts the surprising finding that one can have steady-state reabsorption at low pressures, in contrast to the experiments in Michel and Phillips, if a backward-standing gradient is established in the cleft that prevents the concentration from rising behind the glycocalyx.

endothelial glycocalyx; tight junction; capillary permeability; vesicular transport

STARLING’S CLASSICAL PRINCIPLE for the transcapillary water flow can be described by an equation of the form

$$J_w/A = L_p [P_L - P_T - \sigma_L (\pi_L - \pi_T)]$$  (1)

where $J_w/A$ is the fluid filtration flux across the capillary wall per unit area, $L_p$ is the hydraulic permeability, $\sigma_L$ is the reflection coefficient, and $P_L$, $P_T$, $\pi_L$, and $\pi_T$ are the global values for the hydrostatic and oncotic pressures in the capillary (L) and interstitial (T) compartments, respectively.

Michel (9) and Weinbaum (13) independently proposed that Starling’s hypothesis should be applied locally, just across the thin endothelial glycocalyx layer (EGL), rather than globally, across the entire endothelial layer, between plasma and interstitium, since the EGL is proposed to be the primary molecular sieve for plasma proteins. The primary difficulty in applying the revised Michel-Weinbaum model is that the local Starling forces behind the EGL, $P(0)$ and $\pi(0)$, are spatially varying and unknown because of the large gradients in velocity and protein concentration that are produced by the presence of the tight junction (TJ) strand in the cleft (see Fig. 1A). Typically <10% of the TJ strand is open, with the result that the streamlines and solute flux lines in the cleft depart greatly from one-dimensional (1-D) flow. This creates a highly nonuniform pressure and concentration field throughout the cleft and an important nonlinearity in the resistance to both water and solute, since the presence of the glycocalyx has a substantial effect on the shape of the streamlines and solute flux lines that pass through the discontinuities (orifices) in the TJ strand. The effect on the hydraulic resistance is clearly seen if one were to remove the EGL. The filtration coefficient $L_p$ would rise sharply, not because the integrated average resistance of the glycocalyx is large (typically 10%) but because the EGL diverts the streamlines that would pass directly through the orifice and the resistance of these streamlines is greatly reduced when the EGL is removed. Similarly, there are steep lateral concentration gradients behind the EGL that lead to a significant drop in average concentration across the TJ strand, which depends on the diffusional resistance of the EGL and its thickness. Thus the EGL and the cleft cannot be viewed as two simple linear resistances in series.

In view of the complexity of the detailed flow pattern, Hu and Weinbaum (5) developed a sophisticated three-dimensional (3-D) model with five separate regions to describe the flow geometry depicted in Fig. 1A. This model predicted that the convective flow through the orifice-like breaks in the TJ strand could greatly reduce back-diffusion from the tissue into the lumen side of the TJ strand with the result that the oncotic force behind the EGL could be much smaller than in the tissue at the cleft exit. This prediction is clearly borne out by the experiments in Ref. 4 for frog microvessels and Ref. 1 for rat microvessels in which the tissue is equilibrated with albumin at the same concentration as the lumen. The experiments demonstrate that, at high filtration rates, the proteins on the lumen side of the TJ strand are washed out and nearly the full oncotic pressure $\sigma_L\pi_L$ is felt across the EGL in the case of frog microvessels and ~70% of $\sigma_L\pi_L$ in the case of rat microvessels, although the tissue oncotic pressure is isotonic with respect to the lumen oncotic pressure.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
allows one to obtain solutions that closely mimic the full 3-D solutions for \( J_c / A \) and the net Starling forces across the EGL.

In the pioneering study by Michel and Phillips (10) on individually perfused frog microvessels, these researchers concluded that, at steady state, no reabsorption is possible at low pressure, and they developed a simplified 1-D model to explain this behavior. This model treats the entire endothelium as a uniform fiber matrix layer without clefts where the tissue is at a uniform concentration \( C_i \) given by the ratio of the total solute flux \( J_S / A \) to water flux \( J_W / A \). The latter condition is approximately satisfied in their experiments because the vessels are occluded, the lumen pressure is nearly uniform, and there are no solute sources in the tissue from vesicular transport or leakage through the mesothelium, as in the case of equilibrating the tissue with superfusate. Levick (6) has shown that, for many tissues, this equilibrium value of \( C_i \) in the tissue is \(-0.4\) the lumen concentration \( C_L \). In this paper, we examine what would happen if the tissue were equilibrated at this concentration. The model predicts the unexpected result that one can obtain steady-state reabsorption at low pressure if the far field is clamped at this average tissue concentration. The model also predicts the lumen pressure where the transition from steady-state filtration to reabsorption occurs and shows that a standing concentration gradient conveying solute toward the far field is established in the cleft and that the latter prevents the rise in concentration behind the EGL, which would cut off reabsorption.

A second major contribution of the present paper is that the new model includes vesicular transport and predicts the rise in concentration behind the EGL, which would cut off reabsorption.

AJP-Heart Circ Physiol • VOL 291 • DECEMBER 2006 • www.ajpheart.org
MODEL DESCRIPTION

The 3-D theoretical model for rat mesentery microvessels shown in Fig. 1 A is the same as in Ref. 1 except that the cross-bridging proteins in the cleft have been omitted for simplicity. These linker proteins could be cadherin molecules in the adherens junction, which Shulze and Firth (12) observed just distal to the TJ strand. There could also be linker proteins in other parts of the cleft that do not have an ordered quasi-periodic appearance. One expects that neither the periodically structured TJ strand in the 3-D model, which is treated as a zero-thickness layer, the TJ in Fig. 1 B has a finite depth, LTJ, so that it will provide a finite hydraulic resistance in the 1-D model. LTJ is determined by requiring that the total hydraulic resistance of the cleft be the same as predicted by the 3-D model. The fourth region, region B, is the cleft on the tissue side of the TJ strand whose depth is LTJ = LT. The fifth region, region T, is the tissue space of depth LT and height 2H, the average distance between clefts.

In the 3-D model, the tissue space is divided into two subregions, a near field and a far field. The near field is a region within 5 μm of the cleft exit where the exit jets from the individual junction orifices and adjacent clefts merge with each other and form a uniform flux along the length of the cleft exit. The description of the far field depends on the tissue-loading conditions described at the end of this paragraph. In the 1-D model, we have a continuous narrow slit for the TJ strand, and thus one does not have to deal with the complication introduced by the mixing of discrete exit jets. There is an expansion of the flow from a cleft height 2h to the cleft spacing 2H, but, because LT > 2H, this mixing can be neglected. Similarly, the short mixing regions at the interfaces between each cleft region are neglected in the 1-D model. Thus each region can be approximated by a 1-D convection-diffusion equation. At each interface, we assume that the albumin concentration and albumin flux per unit cleft length are continuous. Three different tissue loading conditions are analyzed. The first is a modified Michel and Phillips (10) model in which the tissue concentration is uniform and given by the ratio of the total solute flux to the total water flux. The second is the tissue loading model in Ref. 1 in which the mesothelium is damaged ~100 μm from the vessel wall and the tissue concentration is set equal to the super fusate concentration at this location. In both cases, the total water and solute fluxes pass through the paracellular pathway. This loading condition is also an approximation for the far-field concentration that is observed in many tissues, where C1 ≈ 0.4 C3 (6). In this case, we require that the tissue concentration approaches this value at 100 μm from the vessel wall. The third loading condition is the model proposed in Refs. 9 and 11 in which transcellular vesicle fluxes of varying strength are added in parallel to the paracellular pathway to see the effect on elevating the tissue concentration and back-diffusion into the cleft.

METHODS

The structural parameters and transport properties for the 1-D and 3-D models for rat mesenteric microvessels are summarized in Tables 1 and 2. All of the other intermediate variables are summarized in the Glossary.
cleft and TJ strand are summarized in Table 1. Analyzing the pressure field for the filtration flow in the 3-D model breaks in its TJ strand. The key insights can be obtained by EGL, in front of a highly nonuniform cleft with discrete orifice-like tance that results from placing a uniform resistance barrier, the first have to examine in more detail the effective hydraulic resis-

Table 1. Structural parameters for rat mesenteric microvessels

<table>
<thead>
<tr>
<th>Structural parameters</th>
<th>Common structural parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGL thickness</td>
<td>( L_E ) 150 nm</td>
</tr>
<tr>
<td>Depth of strand (1)</td>
<td>( L_1 ) 67 nm</td>
</tr>
<tr>
<td>Total cleft depth (1)</td>
<td>( L ) 411 nm</td>
</tr>
<tr>
<td>Cleft height (1)</td>
<td>( 2h ) 18 nm</td>
</tr>
<tr>
<td>Depth of tissue space</td>
<td>( L_{\text{TS}} ) 100 ( \mu ) m</td>
</tr>
<tr>
<td>Length of cleft per unit area of vessel wall (1)</td>
<td>( L_C ) 1000 cm/cm²</td>
</tr>
<tr>
<td>Distance between clefts (1)</td>
<td>( 2H ) 10 ( \mu ) m</td>
</tr>
</tbody>
</table>

3-D model

| Height of TJ break (1) | \( 2d \) 315 nm |
| TJ break spacing (1)   | \( 2D \) 3,590 nm |

1-D model

| Depth of TJ strand   | \( L_{\text{VT1}} \) 1.49 nm |
| Height of TJ opening | \( 2b \) 1.58 nm |

Numbers in parentheses provide reference number. 1-D, 1-dimensional; 2-D, 2-dimensional; EGL, endothelial glycocalyx layer; TJ, tight junction.

3-D Model

Hydraulic resistance. To convert from a 3-D to a 1-D model, we first have to examine in more detail the effective hydraulic resistance that results from placing a uniform resistance barrier, the EGL, in front of a highly nonuniform cleft with discrete orifice-like breaks in its TJ strand. The key insights can be obtained by analyzing the pressure field for the filtration flow in the 3-D model shown in Fig. 1A. The structural parameters for the cleft are taken from Ref. 1 and are summarized in Table 1. The only unknown in the 3-D model, if the EGL thickness \( L_E \) is prescribed, is \( K_P \), which is the Darcy permeability for the EGL. \( K_P \) is then determined by satisfying the measured value of \( L_E = 1.3 \times 10^{-7} \text{ cm s}^{-1} \text{ cm H}_2\text{O}^{-1} \) for rat mesenteric microvessels (1). Using the structural parameters for the cleft in Table 1 and \( L_E = 150 \text{ nm} \), one finds that \( K_P = 9.24 \text{ nm}^2 \).

The detailed pressure and velocity vector profiles for pure filtration for the 3-D model are shown in Fig. 2. These profiles scale linearly with \( P_L \). The structural parameters describing the cleft and TJ strand are summarized in Table 1. \( L_E = 1.3 \times 10^{-7} \text{ cm s}^{-1} \text{ cm H}_2\text{O}^{-1} \) cm·s\(^{-1}\)·cm\(^{-1}\). The pressure behind the EGL is spatially nonuniform. At the centerline, about one-half of the pressure drop occurs across the EGL and one-half occurs in the cleft, whereas at locations toward the edges, \( y = \pm D \), of the periodic unit, the pressure drop across the EGL vanishes and nearly the entire pressure drop occurs across the TJ strand, see Fig. 2A. The pressure drop across the EGL averaged over the entire cleft length is only 10% of the total pressure drop across the endothelial layer. If one naively applies the average pressure behind the EGL as the entrance condition for the cleft, as shown in Fig. 2C, one obtains a much larger water flux than in Fig. 2A (0.034 vs. 0.019 \( \text{cm s}^{-1} \)) for \( P_L = 15 \text{ cm H}_2\text{O} \). This difference arises because the fluid streamline patterns in the cleft for the pressure entrance conditions in Fig. 2. A and C, are dramatically different, as observed in the velocity vector profiles at the cleft entrance, \( x = 0 \), in Fig. 2, B and D. The 3-D model predicts that the removal of the 150-nm EGL, i.e., using \( P_L \) as the entrance condition to the cleft, will lead to a near doubling in \( L_P \). This doubling indicates that approximately one-half of the total pressure drop should occur across the EGL in the region above the orifice. The pressure profiles in Fig. 2A show that this is indeed the case and that the presence of the EGL has doubled the hydraulic resistance on these central streamlines, which provide most of the flow.

The contribution of the EGL to the total resistance has two components. The first component is the intrinsic resistance of the water flowing through the matrix. This is equivalent to the resistance of the matrix in a 1-D flow. The second component is due to the ability of the EGL to change the streamline pattern in the cleft and thus the resistance of the flow after it has passed through the matrix layer. The effect of the streamline pattern change at the cleft entrance on the flow in the EGL itself is minor. The streamlines passing through the EGL are nearly straight because the length of the periodic unit, \( 2D = 3,590 \text{ nm} \), is much greater than the thickness of the layer, \( L_P = 150 \text{ nm} \). Thus the pressure gradient in the \( y \) direction is less than in the \( x \) direction in the EGL. However, once the flow has passed through the EGL, the pressure distribution at the cleft entrance markedly changes the shape of the streamlines as they converge on the orifice, as shown in Fig. 2, B and D. Therefore, when converting the 3-D model to a 1-D model, one has to add a resistance, \( R \), at the back of the EGL to account for the change in streamline pattern that results from adding an EGL of specified thickness or resistance in front of the cleft entrance. This is achieved by defining a corrected pressure, \( P(0)_C \), to

Table 2. Transport parameters for rat microvessels

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin radius</td>
<td>3.5 nm</td>
</tr>
<tr>
<td>Fluid viscosity of water at 37°C</td>
<td>( 700 \times 10^{-6} \text{ Pa s} )</td>
</tr>
<tr>
<td>Hydraulic permeability (1)</td>
<td>( 1.3 \times 10^{-7} \text{ cm s}^{-1} \text{ cm H}_2\text{O}^{-1} )</td>
</tr>
<tr>
<td>Diffusion coefficient for albumin in the lumen</td>
<td>( D_a ) 9.29 ( \times 10^{-7} \text{ cm}^2\text{s}^{-1} )</td>
</tr>
<tr>
<td>Diffusive permeability for albumin in the entire endothelial layer</td>
<td>( P_a ) 0.5 ( \times 10^{-7} \text{ cm}^2\text{s}^{-1} )</td>
</tr>
<tr>
<td>EGL</td>
<td></td>
</tr>
<tr>
<td>Diffusion coefficient for albumin</td>
<td>( D_E ) 0.03% ( D_1 )/0.1% ( D_L )</td>
</tr>
<tr>
<td>Reflection coefficient for albumin (1)</td>
<td>( \sigma_T ) 0.94</td>
</tr>
<tr>
<td>Retardation coefficient for albumin (1)</td>
<td>( \chi_T ) 0.06</td>
</tr>
<tr>
<td>Darcy permeability</td>
<td>( K_P ) 9.24 \text{ nm}^2</td>
</tr>
<tr>
<td>Cleft</td>
<td></td>
</tr>
<tr>
<td>Diffusion coefficient for albumin for regions A and B (5)</td>
<td>( D_{cA} ) 2.0 ( \times 10^{-7} \text{ cm}^2\text{s}^{-1} )</td>
</tr>
<tr>
<td>Effective diffusion coefficient for albumin for the TJ strand</td>
<td>( D_{cT} ) 0.53% ( D_c )/0.35% ( D_c )</td>
</tr>
<tr>
<td>Reflection coefficient for albumin</td>
<td>( \sigma_C ) 0.197</td>
</tr>
<tr>
<td>Retardation coefficient for albumin</td>
<td>( \chi_C ) 0.803</td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
</tr>
<tr>
<td>Diffusion coefficient for albumin</td>
<td>( D_T ) 2.2 ( \times 10^{-7} \text{ cm}^2\text{s}^{-1} )</td>
</tr>
<tr>
<td>Retardation coefficient (5)</td>
<td>( \chi_T ) 1</td>
</tr>
</tbody>
</table>

Numbers in parentheses provide reference number.
account for the streamline pattern change in the cleft due to the presence of the EGL. The flow across the EGL is given by

\[
\frac{u_F}{H_{11005}} = \frac{K_P}{H_{9262}} \frac{P(0)}{H_{20849}} \frac{L_F}{H_{11002}} \frac{P}{H_{20849}} \frac{0}{H_{20850}} \frac{C}{H_{20852}} \left( 2 \right)
\]

where \(u_F\) is the average velocity in the EGL over the cleft entrance, \(P(0)\) is the average pressure behind the EGL, shown in Fig. 2B, and \(R\) is the extra resistance that is required to describe the change in the streamline pattern due to the presence of the EGL and \(\mu\) is the fluid viscosity. The results in Figs. 2 and 3 show that for \(L_F = 150\) nm

\[
P(0)_C = \frac{P_1}{2}
\]

since \(L_F\) in Fig. 3 is reduced by a factor of two for this EGL thickness.

For \(L_F = 1.3 \times 10^{-7}\) cm \(\cdot\) s\(^{-1}\) \(\cdot\) cm H\(\text{2}O\)^{-1}, one finds that \(R = 1.735\) nm \(\cdot\) s\(^{-1}\) \(\cdot\) cm H\(\text{2}O\)^{-1} for a 150-nm-thick EGL whose \(K_F\) is the same as in the 3-D model. \(R\) for other EGL thicknesses can be estimated from Fig. 3 by using the increase in \(L_F\) that occurs when the EGL is removed.

**Diffusional resistance.** Similar to the approach just described for pure filtration, in which we require the hydraulic resistance in the 1-D and 3-D models to be the same, we also require that the two models have the same diffusional resistance in the pure diffusion limit. In this limit, there is no convective flow, and we obtain solutions for the 3-D model that parallel the calculations for the hydraulic permeability, \(L_p\), shown in Fig. 3 for the pure filtration model.

To our knowledge, there are no direct measurements of the diffusion coefficient for albumin in the EGL of rat mesenteric microvessels. Hu and Weinbaum (5) and Hu et al. (4) estimated its value for frog mesenteric microvessels by requiring that their 3-D model predictions provide an optimum fit of the steady-state filtration profile obtained in Michel and Phillips' experiment (10). They predicted that \(D_F\) would need to be \(\leq 0.001\) \(D_L\) \((P_d \leq 1 \times 10^{-7}\) cm/s\), if there was

\[
L_{x0} = 1.3 \times 10^{-7}\text{cm/s/cm H}_2\text{O}
\]

Fig. 3. 3-D model predictions for \(L_{x0}\) as a function of \(L_F\). Predicted \(L_{x0}\) are based on the measured structural parameters described in the text and listed in Table 1 for rat mesentery. \(K_F = 9.24\) nm\(^2\).
to be a sharp bend in the steady-state $J_V/A$ curves in the vicinity of $f/H_9268$, $L/H_9266$, and at the cleft exit when there is an intact EGL whose thickness is 30 nm (A), 150 nm (B), and 500 nm (C). $D_f$ ratio of the average concentration drop across the TJ, $\Delta C_{TJ}$, to the average concentration drop across the entire cleft, $\Delta C_{cleft}$, as a function of $L_F$.

Figure 4, A–C, shows the dimensionless albumin concentration profiles in the lumen, at the cleft entrance ($x = 0$), in the lumen and tissue sides of the TJ strand ($x = L_1$), and at the cleft exit when there is an intact EGL whose thickness is 30 nm (A), 150 nm (B), and 500 nm (C). $D_f$: ratio of the average concentration drop across the TJ, $\Delta C_{TJ}$, to the average concentration drop across the entire cleft, $\Delta C_{cleft}$, as a function of $L_F$.

In the present calculations, we use a 3-D pure diffusion model to predict $D_f$ and require that a specific value of $P_d$ be satisfied. One finds that, if the EGL thickness is 150 nm and $D_f = 0.03\% D_L$, then $P_d = 0.5 \times 10^{-7}$ cm/s. Because the perfusate is always dilute, $D_L$ is approximately equal to the free diffusion coefficient for albumin, which is $9.29 \times 10^{-7}$ cm$^2$/s, predicted by the Einstein relation for $D_L$.

The presence of the infrequent orifice-like breaks in an otherwise impermeable TJ strand leads to a highly nonuniform concentration distribution behind the EGL, which is very similar to the nonuniform pressure distribution behind the EGL obtained for the case of pure filtration. Therefore, $P_d$ does not scale linearly with $D_f$ because the shape of the solute flux lines changes with the thickness of the EGL, much like fluid streamlines.

Michel and Phillips (10) suggested that the EGL is the primary resistance to albumin transport. The 3-D pure diffusion model predicts that, for an EGL whose thickness is 150 nm, 75% of the total diffusional resistance across the entire endothelial layer resides in the EGL if $P_d = 0.5 \times 10^{-7}$ cm/s, whereas only 56% of the total diffusional resistance resides in the EGL if $P_d = 1 \times 10^{-7}$ cm/s and $D_f = 0.1\% D_L$. For this reason, we have chosen $P_d = 0.5 \times 10^{-7}$ cm/s for the present calculations, unless otherwise indicated.

Figure 4, A–C, shows the dimensionless albumin concentration profiles in the lumen at the cleft entrance, on the lumen and tissue sides of the TJ strand, and at the cleft exit when there is an intact EGL whose thickness is 30 nm (A), 150 nm (B), and 500 nm (C). $D_f$: ratio of the average concentration drop across the TJ, $\Delta C_{TJ}$, to the average concentration drop across the entire cleft, $\Delta C_{cleft}$, as a function of $L_F$.

1-D Model

Water velocity. In the 1-D model, continuity of water flux at the interface between each region requires that

$$P_d = 0.5 \times 10^{-7} \text{ cm/s for } L_F = 150 \text{ nm}$$

$$P_d = 1 \times 10^{-7} \text{ cm/s for } L_F = 150 \text{ nm}$$
where $u_i$ is the velocity in the EGL averaged over the entrance area of the cleft, including the fiber and void phases. Therefore

$$u_f = u_A = u_g = b/h \cdot u_{TJ} = H/h \cdot u_T \tag{8}$$


**Depth of tight junction.** The ultrastuctural measurements in Ref. 1 show that $\sim 9\%$ of the TJ strand is open ($2d = 315 \text{ nm}$ vs. $2D = 3,590 \text{ nm}$). To satisfy the condition $2b = 2h \cdot d/D$, which preserves the TJ transport area, we find that the slit height $2b$ is $1.58 \text{ nm}$ or $\sim 9\%$ of the cleft height, $2h = 18 \text{ nm}$.

The average velocity for a parabolic laminar flow in a channel of depth $L_{TJ}$ and height $2b$ is

$$u_{TJ} = \frac{(P_{T1} - P_{T2})b^2}{3\mu L_{TJ}} \tag{9}$$

where $P_{T1}$ and $P_{T2}$ are the pressures at the entrance and exit of the TJ slit.

Likewise, in regions $A$ and $B$

$$u_A = \frac{[P(0)_{Lc} - P_{T1}]b^2}{3\mu L_c} \tag{10}$$

$$u_B = \frac{(P_{T1} - P_{T2})b^2}{3\mu(L - L_1 - L_{TJ})} \tag{11}$$

where $P_T$ is the pressure in the tissue. We assume that $P_T = 0$.

For pure filtration without albumin in the lumen or in the tissue, one has

$$J_{0}/A = L_p (P_L - P_T) \tag{12}$$

$$J_{0}/A = u_A \cdot L_c \cdot 2h \tag{13}$$

$L_c$ is the length of cleft per unit area of vessel wall. Therefore, $L_c = 2h$.

Solving Eqs. 3 and 8–13 when $L_p = 1.3 \times 10^{-7} \text{ cm}^2 \text{s}^{-1} \text{cmH}_2\text{O}^{-1}$, one finds that $L_{TJ} = 1.49 \text{ nm}$. One notices that the depth of region $A$, $L_1$, for the 1-D model is the same as in the 3-D model and that because the TJ strand depth, $L_{TJ}$, is much smaller than the cleft length, the depth of region $B$ in the 1-D model, $L - L_1 - L_{TJ}$, is close to its value in the 3-D model.

One notes that a small channel of height $2b = 1.58 \text{ nm}$ and depth $L_{TJ} = 1.49 \text{ nm}$, which satisfies the measured $L_p = 1.3 \times 10^{-7} \text{ cm}^2 \text{s}^{-1} \text{cmH}_2\text{O}^{-1}$ in rat mesentery, will not allow the passage of albumin (diameter 7 nm). However, in reality, the pore in the TJ strand is $18 \text{ nm}$ high and $315 \text{ nm}$ wide, and our narrow continuous TJ slit is an artificial construct that preserves the TJ area for albumin diffusion. The full cleft height $2h$ is used to estimate the reflection coefficient in the TJ strand and cleft regions $A$ and $B$ in the next section.

**1-D convection-diffusion.** The governing equation for 1-D convection and diffusion in the EGL or any cleft region is given by

$$-D_i \frac{d^2C_i}{dx^2} + (1 - \sigma_i)u \frac{dC_i}{dx} = 0 \tag{14}$$

where $i = F, A, TJ$, or $B$. Solving Eq. 14 one finds

$$C_i(x) = G_{i1} + G_{i2} \cdot \exp \left[ \frac{(1 - \sigma_i)u \cdot x}{D_i} \right] \tag{15}$$

where $G_{i1}$ and $G_{i2}$ are unknown constants that can be determined by applying the boundary and matching conditions in all the regions.

Therefore, the solute flux across any region $i$ per unit cleft length is

$$(J_i)_f(x) = A_i \cdot \left[ -D_i \frac{dC_i}{dx} + \frac{(1 - \sigma_i)u \cdot C_i}{D_i} \right]$$

$$= A_i \left[ -D_i \frac{(1 - \sigma_i)uG_{i2}}{D_i} \exp \left( 1 - \sigma_i \frac{u \cdot x}{D_i} \right) \right]$$

$$+ \frac{1 - \sigma_i)u \left[ G_{i1} + G_{i2} \cdot \exp \left( 1 - \sigma_i \frac{u \cdot x}{D_i} \right) \right] \tag{16}$$

where $A_i$ is the cross-section area of each region and can be replaced by just the height of each region. The reflection coefficient for albumin in the EGL, $\sigma_{iA}$, is abbreviated as $\sigma_i$. A typical value is $0.94$ for rat mesenteric microvessels (1). One assumes that, in the 1-D model, the reflection coefficients for albumin in the cleft, $\sigma_{iC} = \sigma_{iTJ} = \sigma_{iB}$, since in a real cleft in rat mesenteric microvessels the height of the TJ is the same as that in the wide part of the cleft. To estimate its value, $\sigma_{iC} = \sigma_{iA} = \sigma_{iTJ} = \sigma_{iB}$, we use the approximate formula for steric exclusion of a solute of radius $a$ in a channel of half height $h$ (3)

$$\sigma_C = 1 - \frac{3}{2} \left( 1 - \frac{a}{h} \right) + \frac{1}{2} \left( 1 - \frac{a^3}{h^3} \right) = 1 - \frac{3h}{2} + \frac{h^3}{2} \tag{18}$$

where $\chi_C$ is the retardation coefficient in the cleft and $v(x)$ is the local velocity in a parabolic profile. Equation 17 is the definition of the reflection coefficient for filtration defined in Ref. 3. For circular pores and parallel-walled channels and spherical rigid solutes, it is the same as the reflection coefficient for sieving (2). This definition has recently been shown to be true for a periodic fiber matrix in Ref. 14. The upper limit of integration $(h - a)$ for the solute flux arises from the steric exclusion due to the finite solute size. After evaluating the integrals in Eq. 17, one finds that

$$C_{i}(x) = G_{i1} + G_{i2} \cdot \exp \left[ \frac{(1 - \sigma_i)u \cdot x}{D_i} \right] \tag{19}$$

$$(J_i)_f(x) = 2b(1 - \sigma_i)u \cdot G_{i1}, \tag{20}$$

in region $A$

$$C_{i}(x) = G_{i1} + G_{i2} \cdot \exp \left[ \frac{(1 - \sigma_i)u \cdot x}{D_C} \right] \tag{21}$$

$$(J_i)_f(x) = 2b(1 - \sigma_i)u \cdot G_{i1}, \tag{22}$$

in region TJ

$$C_{i}(x) = G_{i1} + G_{i2} \cdot \exp \left[ \frac{(1 - \sigma_i)u \cdot x}{D_i} \right] \tag{23}$$

$$(J_i)_f(x) = 2b(1 - \sigma_i)u \cdot G_{i1}, \tag{24}$$

and in region $B$

$$C_{i}(x) = G_{i1} + G_{i2} \cdot \exp \left[ \frac{(1 - \sigma_i)u \cdot x}{D_C} \right] \tag{25}$$

$$(J_i)_f(x) = 2b(1 - \sigma_i)u \cdot G_{i1}, \tag{26}$$
The diffusion coefficients for regions A and B in the 1-D model are the same as those in the wide part of the cleft in the 3-D model. These coefficients are described by the restricted diffusion of a spherical molecule in a parallel-walled channel. The effective diffusion coefficient for the artificial TJ construct in the 1-D model will be described later using the 3-D solutions shown in Fig. 4.

The governing equation in the tissue is

$$-D_T \frac{d^2 C_T}{dx^2} + u_T \frac{d C_T}{dx} = 0$$

Solving Eq. 27 one finds

$$C_T(x) = G_{T1} + G_{T2} \cdot \exp \left( \frac{u_T \cdot x}{D_T} \right)$$

where $G_{T1}$ and $G_{T2}$ are unknown constants that can be determined by the boundary and matching conditions. The solute flux leaving the cleft is

$$(J_{3y})_T(x) = 2H \cdot u_T \cdot G_{T1}$$

Boundary and matching conditions. MODIFIED MICHEL AND PHILLIPS MODEL. At the entrance of the EGL, the albumin concentration is the same as the lumen concentration. Therefore,

$$C_T(-L_x) = C_L$$

and from Eqs. 8 and 19

$$G_{F1} + G_{F2} \cdot \exp \left( \frac{(1 - \sigma C)u_T \cdot (-L_x)}{D_T} \right) = C_L$$

At the interfaces between each region, the solute flux per unit cleft length is continuous. Therefore,

$$(1 - \sigma)G_{F1} = (1 - \sigma C)G_{A1}$$

$$(1 - \sigma)G_{F2} = (1 - \sigma C)G_{A2}$$

$$(1 - \sigma)G_{T1} = G_{B1}$$

At the interfaces between each region, the concentration is the same. Therefore,

$$G_{F1} + G_{F2} = G_{A1} + G_{A2}$$

(31a)

$$G_{A2} \cdot \exp \left( \frac{(1 - \sigma C)u_T \cdot L_1}{D_T} \right) = G_{T2} \cdot \exp \left( \frac{(1 - \sigma C)u_T \cdot L_1}{D_T} \right)$$

(31c)

$$G_{T1} \cdot \exp \left( \frac{(1 - \sigma C)u_T \cdot (L_1 + L_2)}{D_T} \right) = G_{B2} \cdot \exp \left( \frac{(1 - \sigma C)u_T \cdot L_1}{D_T} \right)$$

(31d)

At the cleft exit, the concentration is the ratio of the albumin to water flux. Therefore,

$$C_B(L) = \frac{J_S}{J_W}$$

Using Eqs. 22 and 25, one obtains

$$G_{B1} + G_{B2} \cdot \exp \left( \frac{(1 - \sigma C)u_T \cdot L}{D_T} \right) = 2h(1 - \sigma C)u_T \cdot \frac{G_{A1}}{2h \cdot u_T} = (1 - \sigma C)G_{A1}$$

and from Eqs. 31c and 31d

$$G_{B2} \cdot \exp \left[ \frac{(1 - \sigma C)u_T \cdot L}{D_T} \right] = -\sigma C \cdot G_{A1}$$

(33)

There are eight unknown constants, $G_{i1}$ and $G_{i2}$ ($i = F, A, TJ, and B$), and four unknown water velocities, $u_T$ ($i = F, A, TJ, and B$). The four water velocities can be related to each other by Eq. 8; therefore, they can be expressed in terms of only one unknown velocity, which we choose to be $u_A$. Thus there are nine unknowns. However, there are only eight equations, Eqs. 31a–31g and 33. For the ninth equation, we apply the Starling principle across the EGL

$$u_A = \frac{K_F}{\mu L_F} [P_1 - P(0) - \sigma \tau_1 \cdot (\pi_L - \pi)]$$

(34)

The oncotic force behind the glycocalyx, $\pi(0)$ (cmH2O), is related to the albumin concentration, $C_0$ (mg/ml), by the nonlinear empirical relation in Eq. 4 of Ref. 8. One notes that Eq. 4 of Ref. 8 is for 35°C (rabbit knee experiments). This can be corrected to 37°C for our rat experiments by the Van’t Hoff equation. $C_0$, in turn, can be expressed as a function of $G_{A1}, G_{A2},$ and $u_A$.

The water flow in regions A, TJ, and B can be approximated by the Poiseuille channel flow, whose velocities are described by Eqs. 10, 9, and 11, respectively. Solving Eqs. 8-11, one obtains

$$P(0)_C = 3 \mu_A \cdot \frac{L - L_1}{h^2} + \frac{h \cdot L_1}{b^3} + P_T$$

(35)

One can relate $P(0)$ and $P(0)_C$ with the use of Eq. 2. Therefore, there are two new unknowns, $P(0)$ and $P(0)_C$, but three more equations, Eqs. 2, 34, and 35. In total, there are 11 unknowns: $G_{i1}$ and $G_{i2}$ ($i = F, A, TJ, and B$), one water velocity, $u_A$, and two pressures, $P(0)$ and $P(0)_C$; there are 11 equations (Eqs. 2, 31a–31g, and 33-35). Solving all of these 11 equations simultaneously, one obtains all the unknowns.

ADAMSON ET AL. (1): TISSUE EQUILIBRATING WITH SUPERFUSATE. In our model for tissue equilibrating with superfusate described in Ref. 1, the boundary and matching conditions in the lumen and in the cleft described by Eqs. 31a–31g still apply. However, the tissue is equilibrated with superfusate 100 μm from the vessel wall, where the mesothelium is damaged, and we assume the tissue concentration is the same as in the superfusate. At the cleft exit, the albumin flux and concentration are continuous. Thus

$$G_{B1} + G_{B2} \cdot \exp \left[ \frac{(1 - \sigma C)u_T \cdot L}{D_T} \right] = G_{T1} + G_{T2} \cdot \exp \left( \frac{h u_A \cdot L}{H_D} \right)$$

(36a)

At the tissue loading site, the albumin concentration is prescribed and equal to that in the superfusate $C_i$,

$$C_i(L + L_4) = C_i$$

(37)

and from Eqs. 8 and 28

$$G_{T1} + G_{T2} \cdot \exp \left( \frac{h u_A \cdot (L + L_4)}{H_D} \right) = C_i$$

(36c)

The matching conditions for the water flux at the cleft entrance and in the cleft (Eqs. 2, 34, and 35) still apply. Therefore, there are 13 unknowns: $G_{i1}, G_{i2}$, ($i = F, A, TJ, B,$ and $T_1$), $u_A$, $P(0)$, and $P(0)_C$; there are 13 equations: Eqs. 2, 31a–31g, 34, 35, and 36a–36c. Solving these 13 equations simultaneously, one determines all of the unknowns.

COMBINED TRANSENDOTHELIAL-PARACELLULAR TRANSPORT. In the case of combined transendothelial and paracellular transport, the boundary and matching conditions in the lumen, at the cleft entrance, at the TJ entrance and exit (described by Eqs. 2, 31a–31g, 34, 35, and 36b) are still valid. At the cleft exit, the concentration is continuous,
in this term, that passes from the plasma to the interstitium per unit time and the clefts. Because \( \frac{J}{S} \) can correct for the added diffusional resistance of the TJ strand.

Diffusion limit. Therefore, we require in the 1-D model that is determined by requiring that the diffusional resistance of the cleft in

\[ C_{T}(x) = \frac{J_s}{J_{v}} \]  

and from Eqs. 8, 28 and 39a

\[ G_{T1} + G_{T2} \cdot \exp \left( \frac{h_{a} \cdot L}{H \cdot D_{T}} \right) = 2h \cdot u_{a} \]

\[ (1 - \sigma_{c}) G_{b1} + 2h \cdot K \cdot \left[ C_{L} - G_{T1} + G_{T2} \cdot \exp \left( \frac{h_{a} \cdot L}{H \cdot D_{T}} \right) \right] \]

\[ = \frac{2h \cdot u_{a}}{h \cdot u_{a}} \]

\[ = \frac{(1 - \sigma_{c}) G_{b1} + 2h \cdot K \cdot \left[ C_{L} - G_{T1} + G_{T2} \cdot \exp \left( \frac{h_{a} \cdot L}{H \cdot D_{T}} \right) \right]}{h \cdot u_{a}} \]  

where the vesicular flux is described by the second term in Eq. 38a. In this term, \( K \) is a coefficient, which depends on the vesicular volume that passes from the plasma to the interstitium per unit time and the partition of solute molecules in the vesicular plasma. Note that, in Eq. 38a, the transcellular flux can cross all along the basal membrane of the endothelial cell whose width is \( 2h \), the average separation of the clefts. Because \( 2H >> 2h \), the height of the cleft is neglected in estimating the basal dimension of the transendothelial pathway.

In general, the tissue concentration in the steady state is given by

\[ C_{T}(x) = \frac{J_{s}}{J_{v}} \]  

and from Eqs. 8, 28 and 39a

\[ G_{T1} + G_{T2} \cdot \exp \left( \frac{h_{a} \cdot x}{H \cdot D_{T}} \right) = 2h \cdot u_{a} \]

\[ 2h \cdot u_{a} \]

\[ = \frac{2h \cdot u_{a}}{h \cdot u_{a}} \]

\[ = \frac{(1 - \sigma_{c}) G_{b1} + 2h \cdot K \cdot \left[ C_{L} - G_{T1} + G_{T2} \cdot \exp \left( \frac{h_{a} \cdot L}{H \cdot D_{T}} \right) \right]}{h \cdot u_{a}} \]  

There are 13 unknowns: \( G_{T1}, G_{T2} (i = F, A, T, I, B, R, U), u_{a}, P(0), \) and \( P(0) \). There are 13 equations: Eqs. 2, 31a–31g, 34, 35, 36b, and 39a–39b to determine all of the unknowns.

Effective diffusion coefficient, \( D_{TJ} \), for the TJ strand. The 1-D model is unable to describe the lateral concentration gradients in front of and behind the TJ strand (see Fig. 4, A–C). Therefore, an effective diffusion coefficient, \( D_{TJ} \), needs to be chosen for the 1-D model that can correct for the added diffusional resistance of the TJ strand. \( D_{TJ} \) is determined by requiring that the diffusional resistance of the cleft in the 1-D model be the same as that in the 3-D model in the pure diffusion limit. Therefore, we require in the 1-D model that

\[ (J_{s,D})_{T1} = - D_{C} \frac{dC}{dx} 2h = - D_{C} \frac{\Delta C_{T1}}{L_{T1}} 2h, \]  

\[ (J_{s,D})_{T2} = - D_{C} \frac{dC}{dx} 2b = - D_{C} \frac{\Delta C_{T1}}{L_{T1}} 2b, \]  

and

\[ (J_{s,D})_{B} = - D_{C} \frac{dC}{dx} 2h = - D_{C} \frac{\Delta C_{B}}{L_{T1} - L_{T2}} 2h, \]  

where \( J_{s,DT1}, J_{s,DT2}, \) and \( J_{s,DB} \) are the albumin diffusive fluxes across the TJ strand and across regions \( A \) and \( B \) and \( \Delta C_{T1}, \Delta C_{A}, \) and \( \Delta C_{B} \) are the corresponding average concentration drops across the TJ strand and regions \( A \) and \( B \), respectively. Because \( J_{s,DT1} = J_{s,DT2} = J_{s,DB} \),

\[ \frac{\Delta C_{A}}{L_{T1}} 2h = - D_{C} \frac{\Delta C_{T1}}{L_{T1}} 2b = - D_{C} \frac{\Delta C_{B}}{L_{T1} - L_{T2}} 2h. \]  

Therefore,

\[ \frac{\Delta C_{A} + \Delta C_{B}}{L_{T1}} 2h = - D_{C} \frac{\Delta C_{T1}}{L_{T1}} 2b. \]  

and

\[ \frac{D_{C}}{D_{C} + D_{T1}} \frac{\Delta C_{A} + \Delta C_{B}}{L_{T1} - L_{T2}} 2h = - D_{C} \frac{\Delta C_{T1}}{L_{T1}} 2b. \]  

In Eq. 45, \( \Delta C_{cleft} \) is the concentration drop across the entire cleft and \( \Delta C_{T1}/\Delta C_{cleft} \) is obtained from Fig. 4D for each EGL thickness.

RESULTS

1-D Model Prediction for Water Flux

Figure 5 compares the 1-D and 3-D predictions for the modified Michel and Phillips model with the analytical solution in Ref. 10 (cleft neglected) for steady-state filtration in which the tissue concentration is set by the condition that \( C_{T} = J_{s}/J_{v} \) and \( P_{0} = 0.5 \times 10^{-7} \) cm/s. In this model, where all water and solutes pass through a paracellular pathway, the \( J_{s}/A \) curve bends sharply near \( \sigma_{T1} \), and there is no steady-state reabsorption even at very low lumen pressure. The \( L_{P} \) used in Fig. 5, \( 1.3 \times 10^{-7} \) cm/s cm H\(_{2}\)O \(^{-1} \), is satisfied by both the 1-D and 3-D models. Figure 5 shows that the \( J_{s}/A \) curve for the present 1-D model corresponds closely with the Michel and Phillips model in Ref. 10, where the cleft is neglected, at all pressures. The \( J_{s}/A \) curve for the 3-D model is in close agreement with both 1-D models at pressures below 40 cm H\(_{2}\)O but diverges slightly at higher pressures. However, the maximum differences are <10%. This result shows that the resistance to water and solute fluxes under the EGL can be accounted for using additional hydraulic and diffusional resistances and that the methods to average the oncotic and
described by the analytic solution for diffusion in the 1-D model. It is difficult and inefficient to describe these gradients by numerical solutions of the 3-D model because the length scales for diffusion in the cleft (tens of nm) are so much smaller than the length scales for diffusion in the tissue (tens of μm). The dashed curve in Fig. 7 shows the range over which the 3-D numerical solutions converge. One notes that the deviation between the 1-D and 3-D models is <10% in this range.

Concentration Profiles Predicted When Albumin Concentration in the Interstitium Is Clamped at a Distance From the Perfused Microvessel

Figure 8 shows the theoretical predictions for the concentration profiles for our 1-D tissue model in which the interstitial albumin concentration is clamped at 50 mg/ml (Fig. 8A) and at 20 mg/ml (Fig. 8B) when \( P_d = 0.5 \times 10^{-7} \text{ cm/s} \). The convective and diffusive fluxes at several key interfaces in the cleft are summarized in Tables 3 and 4. As observed in the experiments of Adamson et al. (1), where \( C_L = 50 \) mg/ml and the superfusate is maintained at the same concentration, our 1-D model predicts that the albumin concentration 5 μm from the vessel wall is relatively insensitive to the filtration rate (or the lumen pressure). Our model predicts that, at \( P_L = 60 \) cmH₂O, the albumin concentration at \( x = 5 \mu m \) is \( \sim 0.8 \) C. However, the convective flux of solute through the TJ slit exceeds the backward diffusive flux, with the result that the concentration behind the EGL is only 0.1 C (5 mg/ml). When the superfusate concentration at the tissue-loading site is reduced to 20 mg/ml, one observes in Table 4 a crossover in behavior at \( P_L \) of \( \sim 17 \) cmH₂O. For \( P_L > 17 \) cmH₂O, there is filtration across the EGL, whereas for \( P_L < 17 \) cmH₂O, there is reabsorption. At low lumen pressures, the concentration at the back of the EGL does not rise high enough to arrest the reabsorption (see Fig. 8B). One notices that the concentration gradient in the tissue is very small (Fig. 8B, inset) and that the relative magnitudes of the diffusive and convective fluxes of albumin in the tissue are comparable at both low and high lumen pressures in Table 4. From the modeling point of view,
the transition from a net diffusion gradient into the cleft region in Fig. 8B when the pressure is above 19 cmH2O and $P_d = 0.5 \times 10^{-7}$ cm/s to a net diffusion gradient out of the cleft at lower pressures is poorly described by numerical methods in the 3-D model. The analytic solutions shown in Fig. 8B provide a more accurate description of these gradients. Additional calculations have been performed for $P_d = 1 \times 10^{-7}$ cm/s (results not shown). There is a small shift in the value of $P_L$ for reabsorption to occur from 17 to 15 cmH2O.

The transition behavior is best described by the convective and diffusive fluxes of albumin at the cleft exit in Fig. 9. For $P_d = 0.5 \times 10^{-7}$ cm/s, the direction of the convective flux at the cleft exit changes at $P_L$ of ~17 cmH2O. At $P_L < 17$ cmH2O, the albumin convective flux is from tissue into the cleft; at $P_L > 17$ cmH2O, the albumin convective flux is from cleft into tissue. The direction of albumin-diffusive flux at the cleft exit changes at $P_L \sim 19$ cmH2O. The creation of an outwardly directed standing gradient in the cleft at $P_L = 19$ cmH2O prevents the rise in concentration of albumin behind the EGL and the shut down of reabsorption.

Concentration Profiles When Tissue Concentrations Are Determined Only by Transendothelial Transport

Figure 10 shows the theoretical predictions for the concentration profiles for the modified Michel and Phillips model when $P_d = 0.5 \times 10^{-7}$ cm/s. For all the lumen pressure conditions, there is always a very small concentration gradient in the cleft, ~2% of the concentration gradient across the EGL or less. At high lumen pressures, $P_L = 40$ or 60 cmH2O, the concentrations at the back of the EGL (the cleft entrance) and in the cleft approach the convection limit, $(1 - \sigma_i)C_L$ and are insensitive to the lumen pressure. This result simply reflects the main assumption in this model that the determinants of the concentration distal to the EGL are only the paracellular fluxes of water and solutes and that tissue concentration gradients are negligible. The flat profiles in the cleft in Fig. 10 reflect the fact that there are no diffusion gradients back into the cleft when there is no exchange of water and solutes across the mesothelium or into any sink in the tissue such as a lymphatic. At $P_L = 25$ cmH2O, a typically average capillary pressure, the albumin concentration behind the EGL is 7 mg/ml. The nonlinear effects of convection are dominant for this low value of $P_d$. This convective effect substantially reduces the albumin concentration behind the EGL at the higher filtration pressures.

Concentration When Tissue Concentrations Are Determined by Transendothelial and Paracellular Transport

With the use of our 1-D model for the combined transendothelial-paracellular transport model, the magnitude of the vesicular flux, $K$, is determined by requiring that at $P_L = 25$ cmH2O, a typical average capillary pressure, the tissue concentration, $C_T$, is 20 mg/ml, or 40% of the lumen concentration as estimated in Ref. 6 for a number of tissues. One finds that $K = 3.43 \times 10^{-7}$ cm/s when $P_d = 0.5 \times 10^{-7}$ cm/s. Equation 38b requires that the solute in the tissue is well mixed so that there is no diffusive flux in the tissue. For this value of $C_T$ and $P_d = 0.5 \times 10^{-7}$ cm/s, the vesicular albumin flux is 80% of the total flux through the combined transendothelial-paracellular pathway.

DISCUSSION

There is a growing body of evidence in support of the Michel-Weinbaum model for a revised Starling principle in which the Starling forces are applied across just the EGL rather than across the entire endothelial layer (1, 4, 6, 7, 9, 13). The original objective of the simplified 1-D model for the revised Starling principle in this study was to reduce the computational effort required to evaluate the changes in hydrostatic and colloid osmotic pressures across the EGL in terms of measurable blood to tissue differences in these variables. However, an unexpected outcome of the modeling has been new insights into the importance of gradients of plasma protein concentration in the interendothelial cleft and in the tissue as modulators of transvascular fluid exchange. In the discussion below, we first evaluate the 3-D to 1-D conversion and then outline the use of the model as a basis for the design of new experiments.
Table 3. Albumin and water fluxes per cleft when there is only paracellular pathway for albumin and the tissue is backloaded, $C_L = C_i = 50 \text{ mg/ml}$ (1)

<table>
<thead>
<tr>
<th>$P_v$, cmH$_2$O</th>
<th>1</th>
<th>10</th>
<th>25</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin flux at $x = -L_d$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>0.35</td>
<td>2.72</td>
<td>2.98</td>
<td>0.81</td>
<td>0.026</td>
</tr>
<tr>
<td>Convective flux</td>
<td>0.85</td>
<td>9.88</td>
<td>33.01</td>
<td>71.49</td>
<td>142.0</td>
</tr>
<tr>
<td>Total flux</td>
<td>1.20</td>
<td>12.60</td>
<td>35.99</td>
<td>72.30</td>
<td>142.0</td>
</tr>
<tr>
<td>Albumin flux at $x = 0^-$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>0.37</td>
<td>4.91</td>
<td>21.45</td>
<td>58.26</td>
<td>129.1</td>
</tr>
<tr>
<td>Convective flux</td>
<td>0.84</td>
<td>7.69</td>
<td>14.54</td>
<td>14.04</td>
<td>12.92</td>
</tr>
<tr>
<td>Total flux</td>
<td>1.20</td>
<td>12.60</td>
<td>35.99</td>
<td>72.30</td>
<td>142.0</td>
</tr>
<tr>
<td>Albumin flux at $x = 0^+$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>−9.97</td>
<td>−90.31</td>
<td>−158.6</td>
<td>−115.6</td>
<td>−30.85</td>
</tr>
<tr>
<td>Convective flux</td>
<td>11.18</td>
<td>102.9</td>
<td>194.5</td>
<td>187.9</td>
<td>172.9</td>
</tr>
<tr>
<td>Total flux</td>
<td>1.20</td>
<td>12.60</td>
<td>35.99</td>
<td>72.30</td>
<td>142.0</td>
</tr>
<tr>
<td>Albumin flux at $x = L^-$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>−10.21</td>
<td>−117.8</td>
<td>−385.6</td>
<td>−792.2</td>
<td>−1,412.7</td>
</tr>
<tr>
<td>Convective flux</td>
<td>11.41</td>
<td>130.5</td>
<td>421.6</td>
<td>864.5</td>
<td>1,554.8</td>
</tr>
<tr>
<td>Total flux</td>
<td>1.20</td>
<td>12.60</td>
<td>35.99</td>
<td>72.30</td>
<td>142.0</td>
</tr>
<tr>
<td>Albumin flux at $x = L^+$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>−13.04</td>
<td>−155.1</td>
<td>−548.1</td>
<td>−1,285.5</td>
<td>−2,929.9</td>
</tr>
<tr>
<td>Convective flux</td>
<td>14.25</td>
<td>167.7</td>
<td>584.1</td>
<td>1,357.8</td>
<td>3,071.9</td>
</tr>
<tr>
<td>Total flux</td>
<td>1.20</td>
<td>12.60</td>
<td>35.99</td>
<td>72.30</td>
<td>142.0</td>
</tr>
</tbody>
</table>

See text for abbreviations not defined.

Table 4. Albumin and water fluxes per cleft when there is only paracellular pathway for albumin and the tissue is backloaded, $C_L = 50 \text{ mg/ml}$, $C_i = 20 \text{ mg/ml}$ (6)

<table>
<thead>
<tr>
<th>$P_v$, cmH$_2$O</th>
<th>1</th>
<th>10</th>
<th>17</th>
<th>25</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin flux at $x = -L_d$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>7.39</td>
<td>10.30</td>
<td>9.69</td>
<td>6.52</td>
<td>1.12</td>
<td>0.03</td>
</tr>
<tr>
<td>Convective flux</td>
<td>−25.69</td>
<td>−12.94</td>
<td>5.66E-3</td>
<td>18.98</td>
<td>66.35</td>
<td>141.3</td>
</tr>
<tr>
<td>Total flux</td>
<td>−18.30</td>
<td>−2.64</td>
<td>9.70</td>
<td>25.50</td>
<td>67.47</td>
<td>141.3</td>
</tr>
<tr>
<td>Albumin flux at $x = 0^-$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>1.59</td>
<td>4.75</td>
<td>9.69</td>
<td>20.28</td>
<td>59.18</td>
<td>130.0</td>
</tr>
<tr>
<td>Convective flux</td>
<td>−19.89</td>
<td>−7.39</td>
<td>2.38E-3</td>
<td>5.21</td>
<td>8.28</td>
<td>11.38</td>
</tr>
<tr>
<td>Total flux</td>
<td>−18.30</td>
<td>−2.64</td>
<td>9.70</td>
<td>25.50</td>
<td>67.47</td>
<td>141.3</td>
</tr>
<tr>
<td>Albumin flux at $x = 0^+$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>247.9</td>
<td>96.30</td>
<td>9.67</td>
<td>−44.27</td>
<td>−43.38</td>
<td>−10.93</td>
</tr>
<tr>
<td>Convective flux</td>
<td>−266.2</td>
<td>−98.95</td>
<td>0.032</td>
<td>69.77</td>
<td>110.9</td>
<td>152.3</td>
</tr>
<tr>
<td>Total flux</td>
<td>−18.30</td>
<td>−2.64</td>
<td>9.70</td>
<td>25.50</td>
<td>67.47</td>
<td>141.3</td>
</tr>
<tr>
<td>Albumin flux at $x = L^-$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>124.1</td>
<td>67.97</td>
<td>9.67</td>
<td>−73.79</td>
<td>−258.9</td>
<td>−491.2</td>
</tr>
<tr>
<td>Convective flux</td>
<td>−142.4</td>
<td>−70.61</td>
<td>0.030</td>
<td>99.29</td>
<td>326.4</td>
<td>632.6</td>
</tr>
<tr>
<td>Total flux</td>
<td>−18.30</td>
<td>−2.64</td>
<td>9.70</td>
<td>25.50</td>
<td>67.47</td>
<td>141.3</td>
</tr>
<tr>
<td>Albumin flux at $x = L^+$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>145.5</td>
<td>81.56</td>
<td>9.66</td>
<td>−104.8</td>
<td>−426.3</td>
<td>−1053.0</td>
</tr>
<tr>
<td>Convective flux</td>
<td>−163.8</td>
<td>−84.21</td>
<td>0.038</td>
<td>130.3</td>
<td>493.8</td>
<td>1194.3</td>
</tr>
<tr>
<td>Total flux</td>
<td>−18.30</td>
<td>−2.64</td>
<td>9.70</td>
<td>25.50</td>
<td>67.47</td>
<td>141.3</td>
</tr>
<tr>
<td>Albumin flux at $x = L_L + L$, mg·ml$^{-1}$·nm·s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusive flux</td>
<td>153.0</td>
<td>83.63</td>
<td>9.66</td>
<td>−101.0</td>
<td>−374.9</td>
<td>−800.7</td>
</tr>
<tr>
<td>Convective flux</td>
<td>−171.3</td>
<td>−86.28</td>
<td>0.038</td>
<td>126.5</td>
<td>442.3</td>
<td>942.1</td>
</tr>
<tr>
<td>Total flux</td>
<td>−18.30</td>
<td>−2.64</td>
<td>9.70</td>
<td>25.50</td>
<td>67.47</td>
<td>141.3</td>
</tr>
<tr>
<td>$J_{LV}$ mm/s</td>
<td>−8.56</td>
<td>−4.31</td>
<td>1.89E-3</td>
<td>6.33</td>
<td>22.12</td>
<td>47.10</td>
</tr>
</tbody>
</table>

See text for abbreviations not defined.
First, with respect to water flows across the EGL. Water flows across the EGL. The EGL must serve as the primary molecular sieve across which the effective oncotic pressure is felt. This is satisfied by applying the Starling principle across just the EGL as opposed to the entire endothelial layer.

We do not compare the 1-D model predictions for the average values for the subglycocalyx hydrostatic pressure \( P(0) \) and albumin oncotic pressure \( \pi(0) \) with the 3-D model predictions individually because neither quantity is measurable. On one hand, the combined pressure-opposing filtration behind the EGL \( |P(0) - \sigma(\pi(0))| \) can be evaluated from the filtration rates \( J_i/\lambda \) and the values of the capillary pressure and lumen oncotic pressure. One condition was when tissue gradients were absent because tissue albumin was assumed to be set only by the paracellular fluxes of water and solute, and there was no exchange across the mesothelium [steady-state conditions in the Michel and Phillips model (10)]. Here, the close agreement between the two models shows that the 1-D model is a reasonable simplified model for the revised Starling principle (Fig. 5). It is noted that the albumin reflection coefficient in the wide part of the cleft, \( \sigma_C = 0.197 \), is small but not negligible. This nonzero reflection coefficient results in a small but finite oncotic pressure difference across the interendothelial cleft due to the steric exclusion of solute at the plasmalemmal boundaries of the cleft, an effect that is not present in the Michel and Phillips model (10).

The second example is the case where the interstitial albumin concentration is clamped at the same concentration as the lumen at a distance of 100 \( \mu \)m from the microvessel, and all protein gradients in the tissue maintain the same sign (see Fig. 6). One notes that both the 1-D and 3-D models slightly underpredict the steady-state \( J_i/\lambda \) measurements, but there is a

To evaluate the role of tissue gradients as determinants of the magnitude and direction of transvascular fluid exchange.

**Evaluation of the 1-D Model**

To make the conversion from the complex 3-D to the 1-D model, one has to capture several key features of the 3-D model. These are the resistance due to flow beneath the EGL, the description of appropriate average pressure and colloid osmotic pressure beneath the EGL, the proper description of the diffusional resistance of the TJ strand, and the accurate description of small gradients of plasma protein concentration in the tissue. Each of these topics is discussed below.

**Water flows across the EGL.** First, with respect to water flows, the resistance due to the 3-D distribution of flow through the EGL and the infrequent breaks in the TJ strands must be appropriately described. The key observation made in the model development is that the presence of the EGL increases the resistance to water flow across the endothelial barrier in two ways: 1) the direct resistance of the EGL itself (accounting for only 10% of resistance) and 2) an additional component due to the flow in the narrow channel between the back of the EGL and the TJ strand as the flow beneath the EGL converges on the infrequent TJ breaks. Specifically, in the absence of the EGL, nearly all of the water goes directly through the orifice. In other words, the streamlines or the velocity vectors corresponding to the pressure distribution in Fig. 2C would be straight and close to the breaks in the TJ strand (see velocity vectors in Fig. 2D).

In contrast, in the presence of the EGL, water is shunted to the side and passes through the narrow channel (average width of 67 nm in this model) between the back of the EGL and the TJ strand. This causes a shift in the streamlines from a straight configuration to a broader orifice-like distribution converging on the breaks in the TJ strand. The velocity vectors in Fig. 2B present both of these changes in flow configuration due to the presence of the EGL, a broader distribution and convergence toward the TJ break. It is this streamline shift that is responsible for most of the resistance to water flows due to the presence of the EGL. One can increase \( L_p \) twofold by removing the 150-nm-thick EGL, although the average pressure drop across the EGL is only \( \sim 10\% \) of the total pressure drop across the entire endothelial layer (see Fig. 3). In the 1-D model, one...
close agreement between the predictions of the two models. The maximum discrepancy between the 1-D and 3-D models is no more than 10%. This shows that the 1-D model reasonably describes the EGL and interendothelial cleft transport as well as tissue protein diffusion.

**Diffusion in the cleft and tissue.** To model the effect of the channeling of water flows into infrequent breaks in the TJ strands on convective and diffusive transport of albumin, we require that the average velocity at the continuous TJ slit in the 1-D model be the same as in the 3-D model with TJ discontinuities. This is satisfied by requiring the height of the narrow slit in the TJ strand, 2b, to be $2h/\bar{D}$. This also ensures that the TJ area for albumin diffusion in the 1-D model be the same as in the 3-D model.

The presence of the infrequent orifice-like breaks in an otherwise impermeable TJ strand in the 3-D model leads to a highly nonuniform concentration distribution behind the EGL, which is very similar to the nonuniform pressure distribution behind the EGL obtained for the case of pure filtration. An effective diffusion coefficient for the TJ, $D_{TJ}$, is introduced in the 1-D model so that it is able to describe the average concentration jump across the TJ breaks due to the convergence of the solute flux lines and lateral concentration gradients at the orifice entrance in the 3-D model. This correction enables the 1-D model to account for the drop in average concentration that occurs across the TJ stand in the 3-D model. This is especially important at low lumen pressures where the convective flux is low and the behavior approaches the pure diffusion limit. It is important to emphasize that the 3-D model prediction of the concentration gradient in the $x$ direction in the cleft is not simulated by the 1-D model since it does not allow for the lateral concentration gradients. However, the concentration profiles in the $x$ direction in the cleft of the 1-D model are representative of only the average concentration profiles of the 3-D model, which would be obtained by integrating the 3-D solution over the length of the periodic unit in the $y$ direction.

The small concentration gradient in the tissue is important because the transport area in the tissue space is $\sim 500$ times larger than the cleft area, since $H/\bar{h} \approx 500$. Thus a small gradient in the tissue can result in a large solute gradient in the cleft when diffusive fluxes in the tissue are important. The numerical relaxation scheme in the 3-D model converges slowly when there are very small tissue gradients, whereas the 1-D model does not have this difficulty, since there is an analytical solution.

**Modulators of Tissue Gradients as Determinants of Transvascular Fluid Exchange**

The 1-D model enables one to evaluate several possible distributions of plasma proteins in the tissue under different experimental conditions. This should lead to the design of new experiments that may resolve some of the difficulties in the interpretation of existing experiments. We first note that the model provides a reasonable description of the experiments in Adamson et al. (1) in which it was demonstrated that a significant colloid osmotic pressure was exerted across the EGL even though the albumin concentrations in the lumen and in the tissue were set equal up to 100 $\mu$m distance from the vessel wall (see Fig. 6). The underlying mechanisms to establish and maintain the concentration difference are, first, that the EGL is the primary molecular filter and, second, that the convective flux of the ultrafiltrate with a low protein concentration through the TJ breaks prevents the accumulation of solute beneath the EGL, even though the concentration gradients favor diffusion of albumin from the tissue into this protected space (see Fig. 8A). Thus, in this case, the concentration of albumin in the space just below the EGL ($x = 0$) is always less than in the tissue.

The important new insights are found when the tissue concentration is clamped at 20 mg/ml (see Figs. 7–9). At high capillary pressures, ultrafiltration across the EGL and the convective flux of the ultrafiltrate into the interendothelial cleft and the tissues maintain albumin concentrations beneath the EGL lower than in the tissue, just as described above. Figure 9 shows the contributions of diffusive and convective fluxes to net transport of albumin away from the base of the EGL (positive) or toward the EGL (negative) as a function of the lumen pressure. For pressures above 19 cm H$_2$O and $P_d = 0.5 \times 10^{-7}$ cm/s, convection dominates over diffusion in the tissue and in the intercellular cleft. However, at a lumen pressure close to 17 cm H$_2$O, ultrafiltration ceases because the hydrostatic and oncotic forces across the EGL are balanced. At this point, diffusion alone accounts for all of the transport of albumin away from the base of the EGL. This is possible because the gradients for diffusion behind the EGL and in the tissue are flat near a pressure of 19 cm H$_2$O and are outwardly directed at lower pressures. Figure 9 shows that, at pressures below 17 cm H$_2$O, where there is net reabsorption, the diffusion flux away from the EGL counteracts the convective flux through the TJ slits carrying albumin up to the protected region behind the EGL reducing the rise in solute concentration in this region. As a result, reabsorption continues at a rate only slightly less than expected from the classical Starling balances.

The above discussion shows that modeling of the convective and diffusive fluxes is subtle. The relative importance of diffusion and convection across a membrane is usually evaluated in terms of the Peclet number, which is the ratio of water velocity to diffusion velocity. It is recognized that, when the Peclet number is high ($> 3$), most solute enters the membrane by convection, and the concentration gradients for solute diffusion into the membrane are small. On the other hand, when the Peclet number is low ($< 0.3$), we usually understand that diffusion will dominate. However, this is the case only when the local concentration difference driving diffusion ($\Delta C$) is similar in magnitude to the local solute concentration ($C$). When this is not the case, e.g., when gradients are shallow and $\Delta C < C$, the convective and diffusive fluxes at low Peclet number are very dependent on the magnitude and direction of the ratio $[C/(\Delta C/\Delta x)]$. This is well illustrated in the change in relative contributions of convective and diffusive transport at pressures close to 20 cm H$_2$O in Fig. 8B, where the tissue concentration is clamped at 20 mg/ml 100 $\mu$m from the microvessel. One notes that the magnitude of convective and diffusive fluxes are comparable even at very high lumen pressure, e.g. $P_l = 60$ cm H$_2$O (see Table 4). The Peclet number defined by $(V/A)L_{ji}/D_T$ is only 0.21 and thus is an incorrect measure of the ratio of convection to diffusion of solute.

Michel and Phillips (10) demonstrated that there was no steady-state reabsorption in frog mesenteric microvessels perfused with a lumen concentration of 50 mg/ml and where the superfusate contained no albumin. Those results are inconsistent with the predictions of the present 1-D model in which the
tissue albumin concentration is clamped at some distance from the microvessel at a superfusate concentration of 20 mg/ml. However, the apparent inconsistency would be explained using the 1-D model if the mesothelium in the frog mesentery, under the conditions of the experiments of Michel and Phillips (10), was a tight barrier and the tissue albumin concentration was not clamped at the superfusate concentration. Under these conditions, the albumin concentration in the tissue surrounding the microvessel is determined only by the water and solute fluxes across the microvessel wall. The results described by Michel and Phillips (10) are accounted for if the mesothelium is tight (there is no exchange of water and solutes across this barrier close to the microvessels) and the concentration of albumin in the tissue is close to that predicted by the steady-state ratio of $J_s/J_v$. The flat gradients in Fig. 10 for the revised Michel steady-state model reflect this condition. On the basis of our present model, we predict that Michel and Phillips would have observed steady-state reabsorption if the frog mesothelium was damaged or if there was a sink in the tissue that would prevent the albumin concentration at the base of the EGL from rising. In this case, one would not reach a concentration sufficient to stop reabsorption. Conversely, we predict that it should be possible to reproduce the results of the Michel-Phillips experiment in rat mesentery under conditions where the mesothelium in the rat is not significantly damaged. To further test this idea, it should be possible to modify the results in Fig. 8 in the microvessels of rat mesentery after the mesothelium is disrupted and the concentration of albumin fixed at different levels at varying distances from the microvessel by locally damaging the mesothelium layer. This would allow one to examine the effect of the magnitude of local tissue gradients.

Relevance of These Experiments to Conditions in the Tissue of Intact Organs

In an intact organ, the gradients of plasma protein in the tissue are determined over long time periods by the delivery of water and solute into the tissue by multiple pathways across the endothelial cells (including interendothelial cell junctions and specialized transendothelial cell pathways such as vesicles and fenestrae) and by the removal of water and protein from the tissue (via transport of water back across the endothelial barriers and the removal of water and solutes via the lymphatics). We neglected the contribution of vesicle transport of albumin in the 1-D model when albumin was added to the tissue because the albumin concentrations in the tissue were dominated by diffusion through the damaged mesothelium. Albumin concentrations in the tissue were then established by adjusting the albumin concentration in the superfusate. To link the results to the situation in normal organs, we first show that, in the modified Michel and Phillips model, the normal range of tissue albumin concentrations (between 0.3 and 0.6 of the lumen concentration) can be described by adding a transcellular vesicle flux in parallel with the paracellular pathway as proposed in Refs. 9 and 11, even when the albumin concentration in the cleft behind the EGL falls to much lower values. In mammalian microvessels of skeletal muscle, the albumin permeability coefficient most likely falls in the range 0.5–1 $\times$ 10^{-7} cm/s. These values represent the combined contributions of both vesicle and paracellular pathways. For $P_d = 0.5 \times 10^{-7}$ cm/s, a large fractional vesicular flux equal to 80% of the total albumin flux across the microvessel wall is required to raise the tissue concentration to 40% of the lumen concentration when $P_f = 25$ cmH2O. This will decrease to 62% for $P_d = 1 \times 10^{-7}$ cm/s.

Future investigations could extend this model to evaluate the possibility that lymphatic drainage creates a sink for albumin. It is possible to imagine a case where the flat gradients shown in Fig. 10 in the absence of a sink for plasma proteins are changed to gradients similar to those in Fig. 8 when lymphatic drainage of water and solute is combined with vesicle transport of solute to the tissue in parallel with ultrafiltration across the glycoalyx and interendothelial cleft. Thus an important question for further study is whether lymphatic drainage of albumin and water is sufficient to maintain such gradients. Levick (6) and Michel (9) have already pointed out that, in organs such as the kidney and intestine, where there is steady-state reabsorption, the dilution of plasma protein by fluid reabsorbed across adjacent epithelial membranes contributes to the maintenance of protein oncotic pressure.

ACKNOWLEDGMENTS

This research was performed in partial fulfillment of the PhD dissertation of X. Zhang from the City University of New York. Present address for X. Zhang: The Leni and Peter W. May Department of Orthopaedics, Mount Sinai School of Medicine, New York, NY 10029.

GRANTS

This research is supported by National Heart, Lung, and Blood Institute Grant HL-44485.