St E. Curry

Determinants of Capillary Permeability: A Review of Mechanisms Based on Single Capillary Studies in the Frog

The objective of this article is to examine two complementary hypotheses that, together, describe the role of the intercellular junction in the regulation of capillary permeability. One hypothesis states that the permeability of capillaries with continuous endothelium is modulated by the area available for the diffusion of small solutes in the spaces between adjacent endothelial cells. The second hypothesis states that the entry of solute into the junctional pathway and the diffusion of solute within the junctional pathway are regulated by the size and distribution of a network of fibrous molecules within the wide part of the junction. Transport through the junction may also be modulated by charge and specific chemical interactions between the solute and side chains on the fibrous network.

When evaluating recent research designed to test the two hypotheses, I will draw mainly on experimental data obtained on individually perfused capillaries in frog mesentery and frog muscle. For these capillaries, methods have been developed to measure the permeability properties of the capillary wall under conditions where the area for transcapillary exchange and the forces determining exchange across the capillary wall are directly measured. Many of the problems to be discussed in this review were identified first from experiments on the microvascular bed in whole organs. Studies based on single capillaries enable the mechanisms of exchange across the capillary wall to be studied directly, removing some of the uncertainties that arise when 1) microvessels, having a range of permeability properties, contribute to exchange in a whole microvascular bed, 2) it is not possible to obtain a precise measurement of exchange area, and 3) the forces determining exchange at the capillary wall (hydrostatic pressure difference, solute concentration difference) are estimated indirectly from measurements in large arteries and veins. For a review of the advantages and limitations of single capillary studies see Crone and Levitt, Michel, Curry et al, and Gore. It is noted that barriers to water flow and diffusion in the interstitium are likely to be minimized during single capillary studies where the tissue is highly hydrated. A review of transport in the interstitium is beyond the scope of this article.

Although the hydrophilic pathways for water and small and large solutes are the primary focus of this review, it should be emphasized that these pathways lie in parallel with specialized transcellular and extra-cellular pathways across the capillary wall. For example, about 10% of transcapillary water flow crosses the wall via a pathway that appears to be a transcellular pathway. Furthermore, lipid soluble solutes can cross the capillary wall by dissolving in two cell membranes in series and diffusing across the cell cytoplasm. It has been suggested that lipids may also cross the capillary wall by lateral diffusion within the cell membranes (see Curry and Scow et al for review). Macromolecules may cross the capillary wall by receptor-mediated endocytosis and by uptake into vesicles, which may shuttle across the cell, or exchange their contents with a vesicle population near the abluminal surface. This parallel array of transcellular pathways does not include the growing number of metabolic functions attributed to the endothelial cell that appear to involve uptake and removal of circulating substances from the blood into the endothelial cell, or uptake, removal, and conversion of substances generated within the tissues adjacent to the endothelial cell. We are only at the very beginning of our understanding of the relation between these metabolic functions of the endothelial cells and the transport processes.

Background

The two hypotheses to be examined are a direct extension of the classical pore theory of capillary permeability. Pappenheimer et al estimated that transcapillary fluxes of small molecules in mammalian hindlimb skeletal muscle could be accounted for if less than 0.1% of the total capillary surface area was available for diffusion in the form of water-filled channels. Although the ultrastructure of the intercellular junctions was not known at the time their paper was published, these authors noted that the calculated area of water-filled pathways was consistent with the hypothe-
sis set forth by earlier investigators that the intercellular junctions formed the principal pathway for water and solutes across the capillary wall.\textsuperscript{16} Pappenheimer et al\textsuperscript{13} also showed that larger solutes were restricted in their passage across the capillary wall more than could be accounted for by the decreased mobility of the solute in free solution. They characterized the increased restriction to diffusion as equivalent to that due to transport through cylindrical pores close to 4 nm in radius. The authors suggested that an "intercellular cement" that was assumed to fill the junctional pathways\textsuperscript{16} may be responsible for the selectivity of the capillary wall. The idea of an intercellular cement substance was put aside when no cement substance was found in tissues prepared using conventional fixation methods. The molecular structures that determine the selectivity of the capillary wall were therefore sought in the fine structure of the tight portion of the junction. It is now recognized that specialized techniques are required to study the structures on the endothelial cell surface. Cytochemical methods have established clearly that the endothelial cells have a glycocalyx that covers the luminal surface of the endothelial cell and extends into the junction as far as the tight junction.\textsuperscript{17,18} Glycosaminoglycans have also been demonstrated in the basement membrane of continuous capillaries.\textsuperscript{19} Zand et al\textsuperscript{20} suggest that silver staining of endothelial cell junctions is the result of glycosaminoglycans throughout the junction.

Some Definitions

A practical measure of the selectivity of the capillary wall to a solute is the reflection coefficient ($\sigma$). The reflection coefficient is defined as the ratio of the effective osmotic pressure exerted across a membrane by a solute in solution to the osmotic pressure of the solution across a semipermeable membrane. When the reflection coefficient of a solute has a value close to zero, the solute enters water-filled pathways in the membrane as if the membrane does not discriminate between the solute and water. On the other hand, as $\sigma$ approaches 1, the membrane excludes solute. The magnitude of $\sigma$ is determined largely by the partition coefficient ($\phi$), which is a measure of the solute concentration in the membrane relative to solute concentration in the bulk solution. For a water-filled channel, steric and electrical forces are the principal determinants of $\phi$.\textsuperscript{9} The following relation from Anderson\textsuperscript{21} appears to have quite general application:

$$\sigma = (1 - \phi)^2$$

(1)

To describe the amount of solute that can exchange across a known area of the capillary wall, the solute permeability coefficient ($P$) is measured. The permeability coefficient of a solute is determined by the area of the transcapillary pathway in each square centimeter of capillary wall ($A$), the diffusion pathlength across the capillary wall $\Delta x$, the diffusion coefficient of the solute within the transcapillary pathway ($D_m$), and the partition coefficient ($\phi$).

$$P = \frac{A}{\Delta x} \cdot D_m \cdot \phi$$

(2)

Values of $\phi$ and $D_m$ can be calculated from the pore and fiber matrix theories described and used in several chapters of the Handbook of Physiology on the Microcirculation.\textsuperscript{1,2,9,22} When the size of the pore or the spacing between fibers is large relative to solute size, $\phi = 1$ and $D_m$ approaches the free diffusion coefficient ($D_f$). Equation 2 then reduces to the form

$$P = \frac{A}{\Delta x} \cdot D_f$$

(3)

The hydraulic conductivity ($L_p$) of the capillary wall is determined by the resistance to water flow in water channels, the area $A$, and pathlength $\Delta x$.

$$L_p = \frac{A}{\Delta x} \cdot \frac{K}{\eta}$$

(4)

$\eta$ is water viscosity, $K$ is a specific hydraulic conductivity with dimensions in square centimeters. Its value is characteristic of pathway geometry. Equation 4 reduces to Poiseuille's Law for a cylindrical pore or rectangular slit.\textsuperscript{9}

Fiber Matrix Model

Development of the Model

One of the most compelling reasons to investigate new mechanisms to account for the permeability properties of the capillary wall is a major inconsistency found when pore theory is used to analyze experimental data describing the permeability and selectivity of the capillary wall. The equivalent pore radius that describes the selectivity of the capillary wall is smaller than the equivalent pore radius that describes the hydraulic conductivity and small solute permeability of the capillary wall.\textsuperscript{1,5,22-22} The problem arises in all capillaries with continuous endothelium, including those in skeletal muscle, lung, heart, and frog mesentery. For example, in frog mesentery, the reflection coefficients to albumin and myoglobin are accounted for by an equivalent pore radius of 5.5 nm, but the hydraulic conductivity of the wall is accounted for by an equivalent pore radius 8 nm. In this section I will examine the hypothesis that a network of fibrous molecules associated with the endothelial cell membranes forms the principal molecular filter at the capillary wall.

Table 1 summarizes expressions for the partition coefficient, diffusion coefficient and specific conductivity for a network of uniform fibrous molecules. The partition coefficient is determined by the volume of the network available to the solute: Solute cannot approach fibers closer than one solute radius. The reduction in the solute diffusion coefficient within the matrix is equal to the probability of a collision between the
Curry Determinants of Capillary Permeability

The void volume is one minus the fiber volume. Derivation of each expression in Table 1 is given in the review by Curry. Further applications and extensions of the theory are given by Turner et al and Michel.

Using the expressions in Table 1, Curry and Michel described the measured permeability properties of frog mesenteric capillaries in terms of a matrix consisting of fibers 0.6-nm radius (the mean radius of a sulfated proteoglycan) that occupy 5% of the matrix volume. At these concentrations, less than 10% of the network is available to a solute the size of albumin, and the matrix restricts the movement of albumin to the same extent as a cylindrical pore 5.5 nm in radius. The fibers also exert the same resistance to viscous water flow as a cylindrical pore 8 nm in radius. The fiber model therefore resolves the inconsistency described above. Curry and Huxley extended the analysis. They found that fibers 0.5 nm in radius occupying 4.5% the fiber volume accounted for permeability and selectivity in mammalian hindlimb and lung capillaries. In the heart, fibers 0.3 nm in radius which occupy 3% of the fiber volume described the data. Of interest was the result that fibers 0.3-0.5 nm in radius occupying less than 5% of the network volume described the selectivity of fenestrated capillaries in the gastric wall, but a combination of coarse and fine fibers (possibly representing fibers from the basement membrane) was required to describe the hydraulic conductivity of fenestrated capillaries.

These calculations demonstrated the feasibility of the idea that a matrix of fine fibers is a major determinant of the selectivity of the capillary. The importance of this result is that it directs attention to the study of factors that might regulate permeability by changing the number, size, and distribution of fibers within a molecular network associated with the endothelial cell surface. Such processes are presumably controlled by the endothelial cell. Mechanisms resulting in a change in the chemical composition of the matrix or rearrangement of the matrix that leads to decreased selectivity at the capillary wall are more likely to be discovered by systematically using an analysis based on the fiber matrix model than if the data were analyzed and interpreted in terms of equivalent pore sizes.

Table 1. Expressions for Transport Properties of a Fiber Matrix

<table>
<thead>
<tr>
<th>Expression</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \phi = \exp\left{ - \left(1 - e\right) \left(1 + \frac{a}{r_f}\right)^2 \right} )</td>
<td>Solute flux</td>
</tr>
<tr>
<td>( D_{\text{matrix}} = D_e \exp\left{ - \left(1 - e\right) \left(1 + \frac{a}{r_f}\right)^2 \right} )</td>
<td>Diffusion coefficient</td>
</tr>
<tr>
<td>( K_e = \frac{e^2}{(1 - e)^2} \frac{r_f^2}{20} )</td>
<td>Hydraulic conductivity</td>
</tr>
</tbody>
</table>

a is solute radius, \( r_f \) is fiber radius, \( e \) is void volume.

Other terms are defined in the text.

Albumin Interactions With the Fiber Matrix

The fiber matrix model has been used to investigate the mechanisms whereby plasma proteins maintain the normal permeability properties of the capillary wall. The removal of albumin from the perfusate in a microvascular bed leads to an increase in the hydraulic conductivity of the capillary wall, a decrease in the selectivity to large molecules such as clinical Dextran, and an increased labelling of the vesicles with macromolecules. The effect has been demonstrated in dog hindlimb, rat hindquarters, and lung. Mann has demonstrated the protein effect in the heart but others deny its existence in this organ. The effect has been studied extensively in frog mesenteric capillaries.

The failure to wash albumin from the tissue surrounding capillaries may account for some of the inconsistencies in whole organ experiments.

The increased vesicle labelling and increased permeability of the capillary wall when albumin is removed from the perfusate can be accounted for if it is assumed that albumin modifies the structure of a network of fibrous molecules. Figure 1 shows an experiment to investigate the concentration dependence of the hydraulic conductivity on albumin perfusate concentration in a frog mesenteric capillary. Hydraulic conductivity \( (L_e) \) does not increase significantly as albumin concentration is reduced from control to very low values but increases fourfold during Ringer's perfusion. When albumin is added back to the perfusate, \( L_e \) does not return completely to control values at low albumin concentrations. There is a hysteresis of \( L_e \) on albumin concentration. These observations suggest that albumin is absorbed to structures in the water pathway. Furthermore, adsorbed albumin appears to limit the movement of additional albumin into or out of the pathway.

Figure 1. Hysteresis of capillary hydraulic conductivity on perfusate albumin concentration. Six separate perfusions of a single capillary in frog mesentery were made, starting at a perfusate albumin concentration of 0.1 g/dl. Hydraulic conductivity did not increase significantly until the capillary was perfused with Ringer's alone. After Ringer's perfusion the concentration of albumin in the perfusate that restored capillary hydraulic conductivity to control values was higher than that required to maintain the control value prior to Ringer's perfusion. The logarithmic concentration scale on the abscissa is broken at an arbitrary point below 0.001 g/dl to include a Ringer value.
There is direct evidence that plasma proteins are preferentially associated with the surface of the endothelial cells. Using antibodies to albumin and γ-globulin, Schneeberger and Hamelin demonstrated that these proteins were preferentially associated with lung endothelial cell glycocalyx, the vesicle diaphragms, and the basement membrane. Schneeberger and Hamelin also demonstrated a direct relation between an increase in the amount of albumin at the cell surface and an increase in the amount of macromolecule label within vesicles and the basement membrane. These experiments do not enable a direct estimate of the amount of albumin at the surface. Cationic ferritin is an electron-dense molecule that modifies capillary permeability in a manner similar to that of albumin and provides quantitative data on the amount of absorbed material associated with significant changes in permeability.

Michel and his coworkers have demonstrated that the binding of highly cationic ferritin molecules to the surface of Ringer’s-perfused frog mesenteric capillaries decreases hydraulic conductivity and increases the effective osmotic pressure that macromolecules exert across the capillary wall. When the same capillaries are fixed and thin sections are examined in the electron microscope, cationic ferritin is found distributed as a uniform layer at the cell surface. The ferritin occupies 8.5% of the volume of the layer. Figure 2 is a model of the fiber matrix containing albumin as recently described. Albumin molecules are assumed to be preferentially absorbed to the side chains of proteoglycans, which form part of the endothelial cell glycocalyx. The permeability properties of frog mesenteric capillaries are accounted for if albumin occupies close to 8.5% of the volume of the matrix and is bound to fine fibers (0.6 nm in radius), which occupy 2.5% of the network volume. At these concentrations the albumin–fiber matrix has the same surface area and solute exclusion properties as the matrix consisting entirely of fine fibers described above. Albumin accounts for 40% of the surface area. The fourfold increase in hydraulic conductivity when albumin is removed from the perfusate is accounted for by the decrease in surface area of matrix in contact with water by close to one half. The decrease in selectivity reflects the decrease in the volume of the matrix from which solute is excluded.

**Electrostatic Interactions**

One objection to the model described in Figure 2 is that albumin carries a net negative charge while cationic ferritin carries a net positive charge. The resolution of this apparent inconsistency lies in a more detailed understanding of the nature and distribution of charged sites on the albumin molecule. Michel and coworkers have demonstrated that the interaction of albumin with the capillary wall involves highly cationic arginine groups in the albumin. When arginine groups are chemically modified so that they no longer carry a net positive charge, albumin fails to modify the permeability of the capillary wall. Much more research is required on this model. For example, in addition to occupying space within the matrix, albumin may order the matrix. An ordered matrix is more selective than a random matrix having the same volume fraction of fibers.

These observations also raise important questions about the role of electrostatic interactions in maintaining the stability of the matrix. After albumin is removed from the perfusate, smaller cationic molecules appear to compete for the site of albumin binding to the surface. Large increases in permeability may be accounted for if cationic molecules disorganize or clump the fibers at the cell surface. These observations have also led to the hypothesis that an intact albumin–fiber matrix reduces the accessibility to the endothelial cell surface of cationic molecules and enzymes that may modify membrane glycoproteins.

Electrostatic interactions due to charge on the fibers may modulate permeability. In frog mesenteric capillaries, the positively charged test solute ribonuclease has a permeability coefficient 2–3 times higher than the negatively charged solute α-lactalbumin. Both solutes have the same effective diffusion radius. When the measured permeability coefficients are compared with small solute permeabilities, it is clear that solute size is the principal determinant of permeability.Charge acts as a secondary modulating factor. The difference between the measured permeabilities of α-lactalbumin and ribonuclease may be accounted for if the fibers carry a net negative charge close to 10 mEq/L. This is much smaller than the charge estimated for the glomerular membrane, which is close to 150 mEq/L. Change interactions for macromolecules at the capillary wall in whole organ studies have received much attention between albumin molecules and the side chains of the proteoglycan appears to require positively charged arginine groups on albumin molecule.
attention recently. Studies of blood-to-tissue transport in skeletal muscle and skin (ear chamber), like those described above on single capillaries of frog mesentery, indicate that positively charged solutes have a higher permeability than negatively charged solutes of similar size. These studies conform to the hypothesis that the capillary wall carries a net negative charge in these tissues. Studies of blood-to-lymph transport appear to give results that conflict with this interpretation. When expressed relative to plasma concentrations, cationic probes in lymph from lung and gut have concentrations less than anionic probes of similar size. Furthermore, anionic sulphated dextrans have higher lymph-to-plasma ratios than neutral dextrans in the lung. The resolution of these problems requires further research. One possibility is that there are preferential pathways (i.e., pathways carrying lower net negative charge) for anionic solutes via vesicles or vesicle channels. Another possibility is that blood-to-lymph experiments reflect properties of the interstitial space. The interstitium may act as a chromatography column to modify lymph composition, depending on solute charge and chemical affinity.

The Location of the Fiber Matrix
Experimental Investigations Using Frog Mesenteric Capillaries

The fiber matrix was discussed in previous sections of this review without specific reference to its location within the capillary wall. Provided the matrix forms the principal resistance to water flow and large molecule transport, its properties as a molecule filter will be the same whether most water flow crosses between the endothelial cells or through other channels such as those formed by the coalescence of vesicles to form vesicle channels. An important clue to the location of the matrix is the observation that most of the transcapillary water crosses the capillary wall via the same pathway as small hydrophilic solutes. Measurements of osmotic flows due to concentration gradients of small solutes across the capillary conform to this conclusion in a variety of capillary beds. In the following section I shall examine the hypothesis that the intercellular junctions form the principal pathway for small solutes. Particular emphasis will be placed on studies in frog mesentery because these vessels have permeability coefficients for small solutes which may be described using a simple model in which the intercellular junction is described as a rectangular slit. This model has been described in detail by Crone and Levitt.

The endothelial cells and the junctional ultrastructure of frog mesenteric capillaries are similar to those of other continuous capillaries. However, there are differences between these vessels and mammalian vessels. The capillaries are larger with diameters close to 20 µm because there are approximately 6 endothelial cells per cross section compared with 1–2 in mammalian vessels. The large size makes these vessels suitable for microperfusion experiments. Colloid osmotic pressure and hydrostatic pressure in these vessels are lower than in mammalian capillaries. The permeability of these vessels may be increased by pharmacological intervention. Preliminary experiments have confirmed previous observations that these capillaries do not respond to histamine, but their permeability is increased by the mast cell degranulating agent 48/80 and by the calcium ionophore A23187. In spite of these limitations, these capillaries have the advantage of enabling the development of models of transcapillary pathways that are experimentally testable using modern biophysical and ultrastructural research techniques.

Rectangular Slit Model of the Junction Pathway for Small Solutes

Bundgaard and Frokjaer-Jensen measured the length of the interdigitating line of contact between adjacent endothelial cells (L) to be 1800 cm/cm² in frog mesenteric capillaries. The maximum area available for exchange (A) between cells is L multiplied by the mean distance between cells (W). In frog mesenteric capillaries, the mean width of the intercellular junction is close to 17 nm and the maximum area for exchange is 0.3% of the total capillary surface area.

Equation 3 enables the permeability coefficients to small solute to be predicted, assuming the whole area between adjacent cells is available for exchange and using the measured distance from lumen to tissue along the junction in the frog mesentery 0.78 µm, as the length of the diffusion pathway. The predicted permeability coefficient of the walls of frog mesenteric capillaries is close to 70 x 10⁻⁵ cm/sec for potassium ions and 19 x 10⁻⁴ cm/sec for sucrose in frog mesentery capillaries. These values are remarkably close to the measured mean value of potassium ion permeability of 67 x 10⁻⁵ cm/sec and sucrose permeability 14 x 10⁻⁴ cm/sec. (A correction for the effect of temperature brings the value reported here, measured at 14–16°C, closer to the value predicted for room temperature, 22–24°C. Corrected value is 17 x 10⁻³ cm/sec.) The permeability coefficients were predicted assuming that diffusion in the junction is free diffusion. Paired measurements of small solute permeabilities in single capillaries were consistent with this assumption. These data therefore conform to the hypothesis that the principal determinant of the permeability to small solutes in frog mesenteric capillaries is the very small area available for diffusion between the endothelial cells.

Agreement between estimates of the fraction of capillary surface area available for diffusion between cells and estimates of the fraction of the endothelial cell surface required to account for permeability and electrical resistance in these capillaries is demonstrated further in Table 2. Measured permeability properties of frog mesenteric capillaries may be accounted for if greater than 90% of the line of contact between adjacent endothelial cells is open to a mean width of 17 nm. A corollary of this result is that structures such as the narrow region of the junction must offer little resistance to the diffusion of small ions and solutes. If the
Values of the Area Available for the Diffusion of Solute Expressed as a Percent of Total Surface Area in Frog Mesenteric Capillaries

<table>
<thead>
<tr>
<th>Source</th>
<th>Area</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrastructure of the junctions (assumed open)</td>
<td>0.29%</td>
<td>54, 55</td>
</tr>
<tr>
<td>Measured permeability coefficients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium ion</td>
<td>0.27%</td>
<td>58</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.26%</td>
<td>59</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.24%</td>
<td>59</td>
</tr>
<tr>
<td>Electrical resistance</td>
<td>0.36%</td>
<td>60</td>
</tr>
</tbody>
</table>

The differences in permeability properties between microvessels can be correlated, in part, with the position of the microvessels within the microvascular bed. Venular capillaries usually have higher permeabilities than mid-capillaries or arteriolar capillaries.

The principal mechanism underlying variation in permeability appears to be changes in area available for exchange and the diffusion distance across the wall, not changes in the structure of the open portion of the channel. For example, Michel has shown that the osmotic reflection coefficient of albumin and myoglobin remains constant in frog mesenteric capillaries, even though the hydraulic conductivities of the same vessels varied over greater than a 10-fold range. These results are not explained by a distribution of pore sizes. The mean reflection coefficient would decrease significantly if more water flowed through large pores in capillaries with higher $L_p$. The same conclusion can be drawn from the fact that solute permeability coefficients to small solutes vary in parallel with $L_p$. These observations indicate that the exchange area and or diffusion distance may be modulated independently of the factors determining selectivity. The simplest hypothesis to account for these observations is that the exchange area and the effective diffusion distance within the junction is determined by the structure of the wide region of the junction.1

Experimental Tests of the Junctional Model

Many important questions arise from the observation that there is a close correlation between the measured permeability to small solutes and the structure of the wide portion of the intercellular cleft. 1) Do electron dense tracers for small solute pathways penetrate the whole junction pathway as required if all the pathway is open? 2) What determines the selectivity of the junction pathway? Does the fiber matrix described earlier fill all or part of the junction, or does the tight junction form the primary filter? 3) Do differences in the measured permeability coefficients to small solutes in different capillary beds simply reflect changes in the area available for exchange or are there more fundamental changes in the whole structure of the junction? All of these questions are currently the subject of active investigation using single capillary techniques. The results of these experiments are likely to provide important insights into mechanisms of transcapillary exchange. Current progress on each of these questions is outlined in the following sections, starting with the third question.

Area vs. Geometry as a Determinant of Small Solute Permeability in Proposed Junctional Pathway

Comparison of Frog Mesenteric and Frog Muscle Capillaries

Studies on frog muscle capillaries provide a bridge between the ideas developed above for the frog mesentery and investigations on mammalian muscle capillaries. The ultrastructure of frog muscle capillaries is very similar to that of frog mesenteric capillaries, but in frog muscle capillaries, the permeability coefficients of the capillary wall to small solutes have values that are 1/10 of those in the frog mesentery. The electrical resistance of frog muscle capillary is also about ten times of that in the frog mesentery (see Table 3 for details). These data illustrate that the high permeability of the frog mesenteric capillaries do not reflect a general characteristic of frog capillaries. There are differences between the permeability properties of capillary beds in different organs of the frog. It follows that factors such as vessel size and the lower hydrostatic and osmotic pressure characteristic of the frog microvasculature are not the principal determinants of capillary permeability properties. An understanding of the factors that determine differences in the permeability properties of different capillaries of similar ultrastructure may provide a fundamental insight into the factors regulating permeability.

One possibility to account for the low solute permeability coefficient of muscle capillaries is that all the

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Muscle*</th>
<th>Mesentery*</th>
<th>Reference†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium permeability</td>
<td>8.6–15.0</td>
<td>67</td>
<td>63</td>
</tr>
<tr>
<td>(cm/sec $\times 10^3$)</td>
<td></td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>Electrical resistance</td>
<td>36–23</td>
<td>3–0.95</td>
<td>64</td>
</tr>
<tr>
<td>($\Omega$ cm$^2$)</td>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Hydraulic conductivity</td>
<td>0.75$\dagger$</td>
<td>5</td>
<td>62</td>
</tr>
<tr>
<td>(cm/sec cm H$_2$O $\times 10^7$)</td>
<td></td>
<td></td>
<td>65</td>
</tr>
<tr>
<td>Sucrose osmotic reflection coefficient</td>
<td>0.12</td>
<td>0.12</td>
<td>62</td>
</tr>
</tbody>
</table>

*Range is arteriolar capillary value to venous capillary value where available; otherwise mean value only is given.
†First reference is for muscle, second for mesentery.
$\dagger$Arteriolar and mid-capillaries only.
junctions are effectively open but the effective width of the junction is reduced to about one tenth of that in frog mesenteric capillaries. Another possibility is that only about 10% of the total junction is open, and the open part of the junction has a similar structure to that in the mesentery. To discriminate between these two possibilities, Curry and Frokjaer-Jensen measured the hydraulic conductivity of the capillary wall and osmotic reflection coefficients to sucrose in frog muscle capillaries. The investigators argued that if the width of the junction has a similar structure to that in the mesenteric capillaries, about 10% of the total junction is open, and the open part of the junction was significantly reduced and the structure of the functional pathway was similar in both vessels. The results of these experiments were quite clear cut. The hydraulic conductivity of frog muscle capillaries was one seventh of the mean value measured in frog mesenteric capillaries. In other words, the hydraulic conductivity in muscle capillaries was reduced relative to values in mesenteric capillaries by almost the same ratio as the permeability to small ions (see Table 3). The results conform to the hypothesis that the effective length of open junction in the muscle capillary was significantly reduced and the structure of the junctional pathway was similar in both vessels.

The results of these experiments were quite clear cut. The hydraulic conductivity of frog muscle capillaries was one seventh of the mean value measured in frog mesenteric capillaries. In other words, the hydraulic conductivity in muscle capillaries was reduced relative to values in mesenteric capillaries by almost the same ratio as the permeability to small ions (see Table 3). The results conform to the hypothesis that the effective length of open junction in the muscle capillary was significantly reduced and the structure of the junctional pathway was similar in both vessels.

The results of these experiments were quite clear cut. The hydraulic conductivity of frog muscle capillaries was one seventh of the mean value measured in frog mesenteric capillaries. In other words, the hydraulic conductivity in muscle capillaries was reduced relative to values in mesenteric capillaries by almost the same ratio as the permeability to small ions (see Table 3). The results conform to the hypothesis that the effective length of open junction in the muscle capillary was significantly reduced and the structure of the junctional pathway was similar in both vessels.

The results of these experiments were quite clear cut. The hydraulic conductivity of frog muscle capillaries was one seventh of the mean value measured in frog mesenteric capillaries. In other words, the hydraulic conductivity in muscle capillaries was reduced relative to values in mesenteric capillaries by almost the same ratio as the permeability to small ions (see Table 3). The results conform to the hypothesis that the effective length of open junction in the muscle capillary was significantly reduced and the structure of the junctional pathway was similar in both vessels.

The results of these experiments were quite clear cut. The hydraulic conductivity of frog muscle capillaries was one seventh of the mean value measured in frog mesenteric capillaries. In other words, the hydraulic conductivity in muscle capillaries was reduced relative to values in mesenteric capillaries by almost the same ratio as the permeability to small ions (see Table 3). The results conform to the hypothesis that the effective length of open junction in the muscle capillary was significantly reduced and the structure of the junctional pathway was similar in both vessels.

Comparison of the Mammalian Muscle Capillaries With Frog Muscle Capillaries

The solute permeability coefficients of frog skeletal muscle capillaries are similar to those measured in mammalian heart, lung, and skeletal muscle. For example, in the rabbit heart the permeability coefficient to sodium chloride is $6.4 \times 10^{-5}$ cm/sec (compare with $8.6 \times 10^{-5}$ cm/sec in frog skeletal muscle) and the hydraulic conductivity is $0.7 \times 10^{-7}$ cm/sec cm H$_2$O (compare $0.75 \times 10^{-7}$ cm/sec cm H$_2$O in frog muscle). The permeability coefficients of sodium chloride in skeletal muscle and lung are smaller than those in the heart ($3.4-3.6 \times 10^{-5}$ and $4.7 \times 10^{-5}$ cm/sec, respectively), but measured hydraulic conductivities are also close to $1/2$ of that in the heart. All values from mammalian capillaries are taken from a review by Renkin. More extensive data is given by Crone and Levitt. Because the mean junction width, junction depth, and length of the line of contact between cells in heart skeletal muscle and lung are similar to those in frog muscle and frog mesentery, the permeability data from these capillaries may be accounted for using a junctional model only if a small fraction (5-10%) of the total area for exchange between endothelial cells is effectively open for exchange. A similar conclusion was drawn by Lassen and Trap-Jensen, and Crone and Levitt. The open area represents only 0.02-0.03% of the total capillary area.

Figure 3 shows an experiment to measure the glucose uptake by an isolated exercising skeletal muscle. The maximum uptake ($J_c$) of 100 mg/min may be accounted for if the microvascular permeability coefficient for glucose is $5 \times 10^{-5}$ cm/sec, the capillary sur-
face (A) is 70 cm²/g, and the transcapillary concentration difference of glucose is at least half the arterial glucose concentration (80 mg/100 ml):

\[ J_1 = PA \Delta C \]

The value of glucose permeability falls in the range of measured values for skeletal muscle and may be accounted for if 8% of the total area between adjacent endothelial cells in skeletal muscle is available for exchange.

All the above studies conform to the hypothesis that effective area for exchange of small solutes is determined by the length of the line of contact between adjacent endothelial cells that is effectively open for exchange.

**Fiber Matrix vs. "Tight" Junction as the Molecular Filter in the Junction**

**The Narrow Part of the Junction as a Molecular Filter**

Before evaluating the hypothesis that a fiber matrix containing adsorbed albumin forms the molecular filter within the open portion of junctional pathway, I shall discuss the alternative hypothesis that structures associated with the tight region of the junction form the molecular filter. The selectivity of the walls of capillaries with continuous capillaries may be accounted for in terms of exclusion from cylindrical pores 5–6 nm in radius or from a rectangular slit 7–9 nm wide (Figure 4A; see also Crone and Levitt). The equivalent slit model has received considerable attention in mammalian skeletal muscle and lung capillaries because the tight or narrow region of the intercellular junction has been described in terms of a space 4–6 nm wide. A similar model has been proposed for placental capillaries. Crone and Bundgaard have suggested that the tight region of the junction in frog mesenteric capillaries is the site of the molecular filter. Crone’s model for slit geometry of frog mesentery is shown in Figure 5A.

If the tight region is the site of the molecular filter to intermediate and large solutes, it must also be the site of the principal diffusion resistance to these molecules. This is because most of the fall in solute concentration between capillary lumen and tissue must occur across the principal molecular filter.

Measurements of solute permeability coefficients to solutes of size 0.25–3.5 nm (Figure 6A) in frog mesenteric capillaries are not easy to reconcile with a series model of the junction in which the tight junction is the primary filter. On one hand, the junction model provides an adequate description of small solute permeability coefficients in frog mesenteric capillaries only if the narrow part of the junction offers little resistance to small solute movement. On the other hand, the narrow region forms the principal molecular filter only if it is also the primary diffusion resistance to large molecules. The series model would therefore be a plausible description of both the permeability to small molecules (0.2–0.5 nm) and selectivity to large molecules (>1.5 nm) in frog mesenteric capillaries only under one very restricted condition. This is that the diffusion resistance of the narrow portion of the junction increases so rapidly with molecular size that the narrow region of the junction changes from a low-resistance segment for small solutes (0.2–0.5 nm in radius) to a high-resistance segment for molecules larger than 1.5 nm radius. None of the present models of restricted solute diffusion will account for such a phenomenon.

The solid line in Figure 6A shows the predicted decrease in capillary permeability with increasing molecular size for the rectangular slit model in Figure 5A. The constricted region (8 nm wide) occupies 10% of the junction length. The mean values of measured permeability coefficients of intermediate and large solutes measured in frog mesenteric capillaries are 4–5 times...
smaller than those calculated using the series model. The permeability of the tight portion of the junction accounts for only 20% of the total diffusion resistance. The tight region does not form the primary molecular filter in this model.

The same conclusions concerning the role of the tight portion of the junction as the principal molecular filter can be reached from more direct experimental manipulations of the capillary wall. For example, if the narrow part of the junction is the site of the molecular filter for intermediate size solutes, then experimental manipulations that reduce the resistance to diffusion of solutes in the wide part of the junction should increase the effective osmotic pressure that solutes such as myoglobin exert across the capillary wall. The effect should be similar to the removal of an unstirred layer from the two sides of a selective membrane. To test this idea Curry et al.34 measured the effective osmotic pressure of a myoglobin solution across the walls of single capillaries of frog mesentery when albumin was present in the perfusate, then when albumin was removed from the perfusate. The effective osmotic pressure of the myoglobin solution did not increase as expected if the narrow region was the primary filter. In fact, the effective osmotic pressure of myoglobin (70 mg/ml) was reduced from 19.5 cm H2O when albumin was present in the perfusate to 10 cm H2O when albumin was removed from the perfusate. Because the removal of albumin does not appear to modify the structure of the tight junction, but can be expected to modify diffusion resistance in the wide portion of the junction, these results appear to be inconsistent with the hypothesis that the narrow part of the junction is the principal determinant of selectivity to solutes the size of myoglobin in frog mesenteric capillaries. Structures within the wide portion of the junction that are modified by the presence of albumin must be taken into account in the description of the selectivity of the capillary wall. The same argument applies in capillaries such as skeletal muscle where the junctional architecture appears to be more complex (i.e., only 10% is effectively open). Several investigators have demonstrated a large reduction in the osmotic pressure of dextran when albumin is removed from the perfusate in skeletal muscle capillaries.30,32

**FIGURE 5.** Two models of the molecular ultrafilter at the capillary wall. A. The molecular filter is assumed to be a narrow constriction within the channel. The slit is drawn using mean values of cleft geometry in the frog mesenteric capillary. Similar structures have been proposed for the junctions in mammalian continuous capillaries. The slit is 17 nm wide except for the constriction (7–8 nm wide), which occupies 10% of the cleft depth. B. The molecular filter is assumed to be a network of fibrous molecules. The fiber matrix is contained within the wide part of the channel and consists of fibers 0.6 nm in radius to which albumin molecules are absorbed. The properties of the albumin–fiber matrix are described in the text.

**FIGURE 6.** Comparison of the solute permeability coefficients predicted from the slit model (Figure 5A) and fiber matrix models (Figure 5B) with directly measured values. Data are from frog mesentery. The analysis extends that given in the text and Table 2 for small solute to larger hydrophilic solutes. A. The diffusion resistance of the constricted portion of the slit lies in series with the wide portion of the slit. The model predicts values that are up to five times larger than measured permeability coefficients. B. The fiber matrix is assumed to fill the wide part of the junction. The matrix offers little resistance to the diffusion of small solutes but accounts for the large decrease in permeability as solute size increases. The fiber model does not include the small calculated resistance of the narrow portion of the junction. Data are from Curry, Curry et al., Adamson et al., Huxley and Curry, and Huxley et al. Solute represented are sodium chloride (0.23 nm), sucrose (0.48 nm), Azure C (0.5 nm) patent blue V (0.6 nm), Evans blue (unbound) 0.89 nm, microperoxidase (0.9 nm), Dextran (MW 3400) (1.7 nm), α-lactalbumin (2.0 nm), ribonuclease (2.0 nm), albumin (3.5 nm). The slit model is based on the hydrodynamic theory of Ganatos et al.33
The Fiber Matrix as the Principal Molecular Filter

Figures 4B and 6B illustrate that a fiber matrix that is assumed to fill the wide region of the space between adjacent endothelial cells in frog mesentery accounts for the measured permeability and selectivity properties of these vessels. The matrix properties used to describe these data are very similar to those described in the first part of this article. A junction filled with a fiber matrix (Figure 5B) accounts for permeability coefficients that range over three orders of magnitude. The fiber matrix model, which treats the junction as a uniform molecular ultrafilter, resolves the difficulties introduced by a combination of diffusion resistances in series as in Figure 5A.

The fiber matrix model does not exclude the contribution of the narrow part of the junction to the permeability and selectivity of the capillary wall. This is because the resistance to diffusion of molecules larger than 1.5 nm at the constriction may be similar to the resistance of a similar length of the wide part of the junction filled with an intermediate size solute. The matrix in the wide part of the junction converts a geometrically nonuniform channel to a uniform molecular filter.

An important property of a channel with a uniform hydraulic resistance is that it exhibits no rectification of water flow: The hydraulic conductivity is the same under conditions of net filtration and net reabsorption. The classical Starling relation for fluid exchange incorporates this assumption, and experiments on whole organs and single capillaries conform to this property of a uniform filtration barrier.2 The nonuniform model (Figure 4A) is expected to show flow rectification. This is because solute would accumulate in the wide part of the junction during rapid filtration through a less selective filter in the wide part of the junction. A corollary of this argument is that the capillary wall is likely to show flow rectification under conditions where the fiber matrix has been disrupted. Reduction of \( L_p \), apparently due to nonspecific accumulation of macromolecules in the water pathway, has been reported by Turner et al26 and Michel and Phillips.37

In summary: Experiments on frog mesenteric capillaries demonstrate that transport via the tight or narrow region of the intercellular junction is not necessary to explain the selectivity of the capillary wall. The properties of the capillary wall as a molecular filter may be explained if a fiber matrix fills the wide portion of the junction. This conclusion, therefore, does not rule out the possibility that other pathways without a tight junction, such as a transendothelial channel, can contribute to exchange provided a matrix fills all or part of the channel.

Structure Function Correlations

The observation that 90% of the junction between adjacent endothelial cells must be open to account for small solute permeability coefficients in frog mesenteric capillaries suggests that ultrastructural studies in these vessels may enable a more precise evaluation of the role of the intercellular pathway in water and small solute exchange. Experiments are needed in which an electron-dense tracer for the small solute pathway is introduced into the lumen of a capillary in which the solute permeability or electrical resistance has been measured directly. If the measured permeability falls in a range where at least 90% of the junction must be available for exchange, the junctions must be marked by tracer.

This test is expected to be far more definitive than those that have been carried out to date using tracer probes on mammalian capillaries in muscle, lung, and heart where only 10% of the junction is expected to be open. The usual observation that electron-dense tracers do not penetrate beyond the tight junction is exactly what is expected in more than 90% of samples in these vessels.

It has been argued that microperoxidase, a small heme protein (MW 1200, Stokes radius 1 nm), may be used as a probe of the small pore pathway.11 Experiments in the author's laboratory to measure the passage of microperoxidase across the walls of frog mesenteric capillaries have shown that the permeability coefficient of microperoxidase is only 2% of that of small solutes such as sucrose in the same vessels.84 In other words, the diffusion of microperoxidase into transcapillary pathways is highly restricted. This may mean that microperoxidase is close to the size limit for the tight junction. It may also indicate that other factors such as electrostatic interaction and binding of the solute to the cell surface may be just as important as size in determining the distribution of tracers. There is a need for a small solute tracer whose permeability can be measured directly and shown to fall close to measured values for other small solutes and which may subsequently be located in electronmicrographs.

If the junctional pathway does not account for the required area for exchange in frog mesenteric capillaries, additional pathways must be found. Clough and Michel85 observed a small population of ablumenal vesicles (0.15–0.30% of the estimated 40,000 vesicles per cell) which are labelled with the high-molecular-weight probe ferritin 1–2 seconds after the tracer was applied to the lumenal surface. The labelling was observed at 20°C and at 4°C. If these ablumenal vesicles are connected to both cell surfaces, they may represent patent transendothelial channels33,86 whose structure is not significantly modified by temperature and whose frequency (60–120 channels/cell) corresponds to an exchange area for small solutes of 0.03–0.06% of the cell surface (channel radius 25 nm). These channels would account for approximately 1/3 of the measured solute permeability to small solutes if their average depth was equal to the thickness of an endothelial cell (0.3 μm). The problem with this interpretation is that it is not clear that the labelled vesicles that are located near the ablumenal side of the endothelial cell actually form direct connections to the tissue.87 It is also possible that quite different apparent channel densities would be measured if smaller tracers were used to mark the channels. The problem of estimating channel densities is discussed further below.
I have tried to estimate the frequency of channels$^{86}$ to compare the area available due to channels with the area available in the junction. The only data available gives channel densities of $4 \times 10^6$ channels/cm$^2$ in arterial and true capillaries and $12 \times 10^5$ channels/cm$^2$ in venular capillaries. The data are from Simionescu et al Figure 14,$^{88}$ which reports the distribution of 105 to 115 transendothelial channels formed by fused vesicles in each of 6 segments of the microcirculation. The striking conclusion to be drawn from these calculations is that the observed channel frequency is 50–100 times larger than the frequency required to account for the measured permeability coefficients to small solutes in skeletal muscle capillaries. The calculation assumes the channels are 25-nm radius and cross the endothelial cell (mean thickness 0.3 $\mu$m). The calculations also assume that the diffusion resistance of diaphragms and structures to small solutes and ions is small. It is apparent from these calculations that the channels demonstrated using electron-dense tracers cannot be open channels for most of the time.

It might be argued that transendothelial channels could form by the transient fusion of adjacent vesicles. This hypothesis has been tested by measuring the effect of temperature on the hydraulic conductivity of the capillary wall in several microvascular beds. The rate of formation of channels is expected to decrease at lower temperature if they form by transient fusion due to random thermal motion. This does not appear to be the case. Capillary hydraulic conductivity decreases with temperature almost exactly to the extent expected from the increase in water viscosity.$^{5,90}$ It follows that the structure of the principal water pathway must be stable and insensitive to temperature changes. In contrast, the exchange of macromolecules between vesicles is reduced at low temperature.$^{85}$

**Transport of Macromolecules**

**Pathways for Macromolecule Exchange**

Discussion in this section is restricted to a few controversial topics that may be addressed using single capillary techniques. The transcapillary exchange of macromolecules across the microvascular bed in whole organ studies cannot be accounted for in terms of transport processes through the principal pathways for water. This is because the permeability coefficient for macromolecules larger than albumin do not decrease with increasing molecular size as expected for the selective hydrophilic pathways discussed above. One or more additional pathways (large pores or specialized forms of vesicle transport) must contribute to the flux. There have been major developments in conventional ideas about both these pathways.

Using modern forms of the irreversible thermodynamic relations that describe diffusion in the presence of a superimposed volume flow, several investigators have shown that, at high capillary pressure (high transcapillary water flows), most of the flux of macromolecules larger than albumin is coupled to water flow through a small population of larger pores$^{22,90}$ in the microvascular bed of whole organs. An important problem that has not been addressed in any analyses of whole organ data is whether the “large pore” convective pathway is present in all capillaries.$^{91}$ It may be restricted to venous capillaries or to regions around damaged or dividing endothelial cells. Experiments on individual capillaries in different parts of the microvascular bed are required to clarify these important issues. Methods that enable both diffusive and solvent drag components of transcapillary flux to be measured in single capillaries are being developed.$^{50,82,83,92}$

When hydraulic conductivities and pathway permeability and selectivity properties measured at high capillary pressures are extrapolated to predict macromolecular fluxes at normal capillary pressures, a major discrepancy arises. The measured macromolecular flux is larger than that predicted from the transport properties at high flow.$^{5,90}$ One explanation of this result is that high flows modify transcapillary exchange, making extrapolations to low flow conditions invalid. Another explanation is that transport processes that do not involve diffusion and convection through porous pathways contribute to basal levels of macromolecule exchange. The resolution of these important problems remains for future research. Various forms of the nonporous pathways are discussed by Renkin.$^{90}$

Experiments on single capillaries of frog mesentery have resulted in two important observations that are inconsistent with the conventional views of the role of vesicles in macromolecular exchange. First, vesicles appear to be arranged in racemose clusters associated with the cell surface.$^{86}$ The latter observation is based on serial sections using very thin sections (150 A).$^{87}$ The result presents a fundamental challenge to the concept that vesicles attach to one surface of the endothelial cell, equilibrate with tissue or lumen fluid, detach, and diffuse to the adjacent surface to discharge their contents. Crone and his colleagues interpret these data to show that vesicles are not free to participate in any exchange processes. Clough and Michel$^{13}$ also question the model whereby free vesicles shuttle from one membrane to the other. They have proposed a new model of transport via vesicles.

It is possible that the contents of one vesicle are exchanged with an adjacent vesicle. Successive exchange steps between vesicles within a cluster and between adjacent clusters could thus contribute to transcapillary exchange. An important new observation in the study of vesicle transport is the direct experimental confirmation of exchange of contents between vesicles as demonstrated by the dilution of vesicle contents. The process is described in terms of transient coalescence of vesicles and mixing of macromolecular contents.$^{13}$ By this process solutes are observed to cross the luminal to the abluminal surface of capillaries.

It appears premature to conclude that vesicles do not contribute to macromolecule exchange. However, it is equally clear that the contribution of free “shuttle” vesicles to net exchange is presently undergoing a major reevaluation. For a recent review, the reader is referred to the proceedings of a workshop on endothelial...
These observations focus attention on possible roles played by vesicles. All theories of vesicle transport other than transport (e.g., reserves of cell membrane effective area available for exchange and the structure of the size-limiting barrier within the exchange pathway, are modulated independently by endothelial cells. Taken together the two hypotheses form the starting point for a detailed understanding of the factors that regulate the permeability of capillaries. For example, a variation in the magnitude of the permeability coefficient with no change in the selectivity of the capillary wall to plasma proteins may reflect changes in the area of the intracellular junction that is available for diffusion with no change in the structure of the fiber matrix within the junction. Factors that modulate the size, shape, and volume of the endothelial may act via this mechanism. On the other hand, changes in the selectivity of the capillary wall with no change in the normal ultrastructure of the junction may reflect a redistribution of the matrix within the junction or a change in the charge or chemical affinity of the chemical substances within the junction. Factors that act on the endothelial cell surface may modulate permeability via this mechanism. Very large changes in permeability that may be associated with the physical disruption of a junction may change both permeability and selectivity. The selectivity of the capillary wall may be returned toward normal by a fiber matrix extending to fill the gap long before the total area for exchange is returned toward control.

Acknowledgments

This review was begun during sabbatical leave at the University Laboratory of Physiology, Oxford University. I thank Drs. C.C. Michel and Geraldine Clough for their helpful discussions and critical review of the first draft. I also thank Drs. E.M. Renkin and W.L. Joyner for their criticism of the manuscript.

References

Determinants of Capillary Permeability


44. He P, Curry FE, Huxley VH: Ribonuclease increases capillary permeability only during Ringer perfusion (abstract). Fed Proc 1986;45:1159


KEY WORDS • capillary permeability • single capillaries • endothelial transport • fiber matrix • pore theory • regulation of capillary permeability • transcapillary exchange • frog mesenteric capillaries
Determinants of capillary permeability: a review of mechanisms based on single capillary studies in the frog.

F E Curry

doi: 10.1161/01.RES.59.4.367

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/59/4/367.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/