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REVIEW OPEN ACCESS

Eugene M Renkin. His Many Contributions to Microvascular Research With Examples of How They Inform Current Investigations of Microvascular Dysfunction

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ABSTRACT

Eugene Renkin used simplified uniform models of microvascular exchange units to describe the fundamental functions of the microcirculation: a cylindrical pore to characterize the barriers to exchange of water and solutes; a uniformly perfused capillary to distinguish flow-limited exchange from diffusion-limited exchange; and a membrane with large and small pores to describe macromolecule exchange between blood and lymph. A key idea linking these concepts to microvascular dysfunction is that local blood flows, microvascular pressures, and the permeability of the vascular wall are not uniformly distributed within microvascular beds. Renkin's concept of microvascular clearance of small solute was extended to show how heterogeneity in blood transit times compromised exchange. It was also extended to evaluate the relative contribution of diffusion, convection, and vesicle exchange to microvascular exchange of macromolecules when there is heterogeneity in macromolecule permeability, measured by the presence of large pores. An extension of his analysis to smaller proteins (14–20 KDa) showed that convective transport may limit the diffusion of inflammatory peptides, therapeutic agents, and toxins from the tissue into circulating blood. We include recent examples of the growing understanding of microvascular dysfunction in chronic disease and approaches to modeling heterogeneity in normal and diseased states.

1 | Introduction

This review is dedicated to the memory of Eugene Renkin, who died in November 2022. Gene Renkin was a major figure in the study of microvascular permeability and blood-tissue exchange throughout the second half of the twentieth century. A characteristic of Renkin's research was the critical testing of theoretical models of exchange against experimental data. Starting with simplified uniform models, he identified the basic principles governing microvascular exchange. By applying this approach to reliable and reproducible experimental data, he encouraged further development of such tests and their wider application. We highlight this approach by first giving a brief overview of the scope of his research and then describing key ideas that can be extended to address current research and the growing awareness of the role of microvascular dysfunction in the clinic. The scope of the latter section is limited to the areas that Charles Michel had written or outlined up to the time of his death and

[†]Charles Michel passed away suddenly July 19,2024 during the preparation of this manuscript.

where his own publications are a clear guide to content. They include (i) the effect of changes in blood flow on the exchange of small solutes between blood and tissue and how these are modified by heterogeneity in blood flow distribution; (ii) the regulation of capillary pressure during changes in blood flow and how changes in microvessel pressure modulate microvascular fluid and plasma protein exchange in both small pores and larger pores; and (iii) the exchange of small peptides across the microvessel wall especially when the protein is initially distributed mainly in the tissue. We also highlight some of the research on individually perfused microvessels that Renkin inspired in our own laboratories, including the idea that the endothelial glycocalyx is part of the size-limiting structure in the capillary wall that Renkin described in terms of the restricted diffusion and steric exclusion in small pores.

2 | Renkin's Research Contributions

An outstanding graduate student of John Pappenheimer, Gene Renkin's doctoral thesis included much of the experimental work for the paper of Pappenheimer, Renkin & Borrero in 1951 [1] which introduced the quantitative form of the pore theory of biological membranes. Previous investigators had noted that the capillary wall acted as a molecular ultrafilter, restricting the movement of plasma proteins including albumin but allowing the passage of small solutes and water. When Renkin joined the laboratory, Pappenheimer and Soto-Rivera had just provided impressive evidence for the applicability of the Starling Principle of microvascular fluid exchange by demonstrating that an isolated cat hindlimb neither gained nor lost weight during continuous perfusion when capillary pressure was equal to the colloid osmotic pressure of the perfusate. Renkin extended this approach to measure the magnitude and time course of osmotic flows as small hydrophilic solute diffused from plasma into the tissue. The key observation was that the area available for exchange by diffusion was less than 1% of the total capillary surface area, and that, as solute size increased, this effective area was reduced more than expected from free diffusion. The additional resistance to diffusion was accounted for when the solute was assumed to cross the capillary wall through cylindrical pores or rectangular slits. This is the pore theory of capillary permeability. The presentation of this classical paper with its detailed explanation of the limits of various experimental approaches, critical evaluation of novel osmotic transients experiments and their interpretation using pore theory, provides an insight into the research environment where Renkin received his early training.

The emphasis on theory and experiment is also apparent in the next step in Renkin's research after he was appointed as a research fellow in the Biology Division of the Brookhaven National Laboratories. First, he carried out detailed experiment in artificial porous membrane and successfully described the exchange of water and a range of test solute using pore theory [2]. The theory described diffusion though pores in terms of the extra drag on an idealized molecule (represented as a sphere with a radius determined by a solute's free diffusion coefficient) as it moved within the pore. It also accounted for the reduced area available for diffusion into the pore because a spherical solute was excluded from an area near the wall by a distance equal to its radius. These observations raised the question of whether the molecular structures forming the junctions between endothelial cells could be directly identified as real pores (or narrow intercellular slits) or whether a fiber matrix (at the time described as an intercellular cement) was part of the size-selective ultrastructure of the endothelial barrier.

Renkin then joined the National Institutes of Health as a research associate and began investigations of the effects of changes in blood flow on microvascular exchange of small hydrophilic solute. He also unambiguously defined the concepts of blood-tissue extraction and microvascular clearance of solutes [3, 4]. We examine this work in detail in a separate section because it describes the fundamental role of the microcirculation to deliver nutrients to the body tissue. Furthermore, it is now widely recognized that the idealized state of uniform blood flow to all microvessels in an organ is never achieved and that heterogeneity of blood flow distribution is a major factor limiting microvascular function in multiple disease states, including diabetes, sepsis, and compromised renal and cardiac function. Examples of publications since 2020 are in the Perspective section at the end of this review.

Soon after his appointment to his first academic position at George Washington University, Renkin was invited by the American Physiological Society to give the Bowditch lecture for 1963, an honor to recognize the achievements of an investigator under the age of 40 [5]. Rather than reviewing his major contributions already reported in at least 14 published papers, he chose to analyze recent work published by Grotte and Mayerson et al. [6, 7] on the passage of proteins and dextrans of different molecular weights between the plasma and the regional lymph. Here he demonstrated that the relationships between permeability and molecular size for these macromolecules were quite different from those for small solutes. He showed that the quantitative data of permeability to macromolecules could be interpreted either in terms of a small population of large pores (as Grotte had suggested) or in terms of vesicular transport through the endothelial cells, as suggested by Palade [8]. This led to experimental studies in which Renkin and his colleagues measured the flow and composition of lymph to evaluate blood to tissue exchange of plasma proteins. These studies raised the question of whether net movements of plasma proteins between the plasma and interstitial fluid were entirely convective (via "large pores") or dissipative (via diffusion through large pores or vesicular transport) [9–12]. This topic is also discussed in a separate section where we extend Renkin's analysis to evaluate the relative contributions of convective and diffusive transport through pores as solute size and the balance of hydrostatic and colloid osmotic pressure are changed.

The 1960's and 70's were a period of rapid expansion of investigations of microvascular exchange by Renkin and several other research groups in a variety of organs using both tracer extraction methods and the analyses of lymph. Renkin's research was at Duke University (Department of Physiology and Pharmacology, 1963–1973) and then in the Department of Human Physiology at the University of California, Davis (1974–2001 and Chair 1974–1991). In his 1977 Landis Award Lecture to the Microcirculatory Society, Renkin reviewed this research, comparing morphology and function of microvascular exchange of water, small

hydrophilic and lipid soluble solutes, and larger plasma proteins in microvascular beds with both continuous and fenestrated endothelium [13]. In further experiments using an experimental approach like those being developed in other laboratories [14–16], Renkin used serum albumin labeled with two different isotopes to take into account changes in both the vascular and extravascular distribution of the tracer [17]. The approach was later extended to include investigations in new animal models of exchange, including genetically modified rats and mice [18–20].

3 | The Effects of Increased Microvascular Blood Flow on Exchange

3.1 | Net Transport of Permeable Solutes Between Blood and Tissues

The following example shows Renkin's concept of an idealized capillary unit as the fundamental unit of exchange in the microcirculation [3, 4]. He extended the quantitative analysis of exchange between blood and tissue by Kety [21] to account for the diffusive flux (*Js*) of solutes between blood and tissue. Fick's Law of diffusion is used to account for the loss of a small diffusible plasma solute from a segment of microvessel having a solute permeability coefficient *P* and surface area for exchange *S*.

A mass balance across the segment fractional length ΔX gives:

Flow In = loss due to diffusion + flow out

$$Q \times Cx = PS\Delta X(Cx - Ct) + Q(Cx - \Delta C) \quad (1)$$

where *Cx* is the plasma concentration at distance *x* and *Ct* is the tissue concentration.

The differential form of Equation (1) may be integrated between arterial (*a*) and venous (*v*) sides of the vascular bed:

$$\left(C_v - Ct\right) / (Ca - Ct) = e^{-PS/Q} \quad (2)$$

when *Ct*, the tissue concentration, is zero, the arteriovenous concentration difference can be expressed as a function of blood flow and arterial concentration:

$$C_a - C_v = C_a \left(1 - e^{-\frac{PS}{Q}}\right) \quad (3)$$

Renkin introduced the term Extraction, E which measures the a-v difference in Equation (3) as a fraction of C_a when solute is moving from blood to tissues, that is, *E =(Ca-Cv)/Ca*.

Renkin also defined the term “Clearance” as related to microvascular exchange. Clearance (*Js/Ca*) is the volume of plasma from which solute is lost as it crosses the microvascular barrier.

$$\text{Clearance} = Js / Ca = Q * E = Q * \left(1 - e^{-\frac{PS}{Q}}\right) \quad (4)$$

Figure 1 shows values of the clearance of potassium-42 in skeletal muscle as a function of blood flow superimposed on the theoretical curves for various values of the permeability surface area product *(PS)* from Equation (4) [22, 23]. For potassium-42 transport into resting muscle, clearance is approximately described by the theoretical curve with *PS* of 5 mL/min per 100 g tissue. With

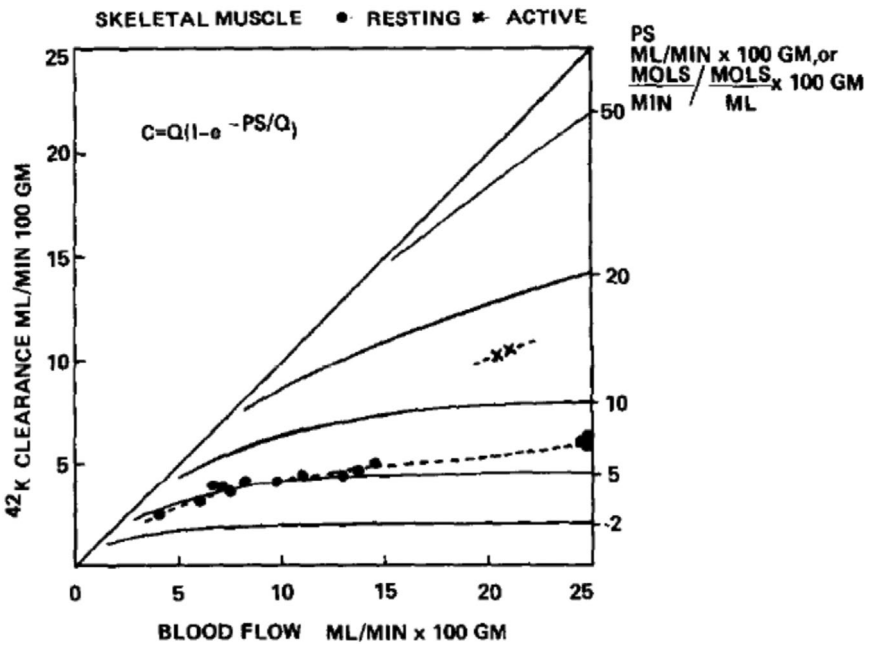

FIGURE 1 | The Figure from Renkin's B. W. Zweifach lecture describes the relation between solute clearance and blood flow according to Equation (4). The solid lines are for PS values for solutes of increasing permeability as recorded on the right side of the graph. For values of *PS/Q* less than 2, clearance becomes independent of flow and the exchange is diffusion limited. On the other hand, for values of *PS/Q* greater than 20, the relation between clearance and flow approaches a linear relation and the exchange is flow limited. At intermediate values of *PS/Q*, both blood flow and barrier permeability determine clearance. The solid points are measured values of clearance of potassium 42 as blood flow is increased in a muscle at rest. The crosses are for active muscle contraction. The key assumption in the derivation of each theoretical curve is that *PS/Q* is the same for all exchange microvessels. Reproduced with permission from 23.

active muscle contraction, the clearance increases more than two-fold and falls near a curve with *PS* between 10 and 20 mL/min per 100 g. The increase in clearance for the active state due to metabolic vasodilation is accounted for by the opening of additional exchange vessels to perfusion. This is supported by the observation that the fractional increase in PS is similar to the increase in the value of the filtration capacity (*LpS*) measured by Cobbold et al. [24]. Thus, to a good approximation, the increased surface area for small solute exchange is similar to that for transvascular water exchange. Renkin noted that a key assumption is that the distribution of blood flow to all vessels is uniform so that *PS/Q* is constant. This assumption is never likely to be valid, but he suggested that mechanisms such as an increase in permeability as flow increases may function to match the local *PS* value to local blood flow. The link between these classic observations and current interest in the role of microvascular dysfunction with respect to exchange is reviewed below.

3.2 | Heterogeneity of Blood Flow in the Microvasculature Under Normal and Diseased States

The more detailed understanding of the relation between blood flow and exchange was developed in Renkin's Zweifach Award Lecture [23] and the comprehensive chapter on the Control of the Microcirculation in the Handbook of Physiology: Microcirculation [25]. The broken line in Figure 1 demonstrates one example of the systematic deviation of the measured clearance from the simplified model leading to Equation (4). Here blood flow was increased passively in resting muscle by raising perfusion pressure. *PS* values for small solutes calculated from measured clearances fell below the theoretic curve at low flows but increased relative to the mean as blood flow increased. One common explanation is that additional capillaries open as flow increases, as in the case of active contraction with metabolic vasodilation. However, Renkin argued that this cannot account for variation in PS values under these conditions. Unlike the case of active contraction, the increases in PS do not track with an independent estimate of exchange area from the filtration capacity (*LpS*) which is not increased. Also, the dependence of *PS* on flow is different with solutes of different sizes. He argued that the increase in apparent PS is expected if the effective pathlength or transit time is different in different parts of the vascular bed, as expected when there is an uneven distribution of blood flow between open microvessels. Rather than interpret the theoretical line in Figure 1 as different perfusion conditions (rest versus active contraction in the whole vascular bed), Renkin noted that they can be imagined as describing the distribution of blood flows and *PS* values within a perfused organ. The curves in Figure 1 can then represent the range of possible ways *PS/Q* may vary. Using a relation derived by Bass and Robinson [26], Renkin demonstrated that *PS* would fall from 7 mL/m per 100 g at the highest flow to as low as 3 mL/min/100 g at the lowest flow measured when the standard deviation of values of *PS/Q* about a mean was in the range 0.7 to 0.9. The key observation that heterogeneity of flow results in a significant decrease in microvascular exchange is now recognized as the mechanism resulting in microvascular exchange dysfunction in multiple disease states. McClatchey et al. [27] examined flow distributions caused by factors as varied as low perfusion pressure due to cardiovascular disease, blockage of microvessels in inflammation (particularly sepsis), and modified local microvascular control (including damage to the glycocalyx) [28]. The development of network models of blood flow and exchange is an important example of the extension of the idealized flow unit (see Perspectives Section).

3.3 | Regulation of Microvasculature Flow and Pressure. Microvascular Dysfunction

Renkin's chapter on Control of the Microcirculation in the Handbook of Physiology [25] reviewed the general principles that govern the interaction between the control of blood flow and changes in microvascular pressure. A key insight derived from a simplified lumped model of a microvascular bed is that, whereas blood flow to tissue is determined by the sum of the resistance to blood flow on the arterial side of the microvasculature (r_a) and the venous side of the circulation (r_v), the mean microvascular pressure (*Pmc*) is determined by the ratio, *ra/rv*.

Specifically:

$$Pmc = \frac{P_a + P_v(r_a / r_v)}{1 + r_a / r_v} \tag{5}$$

Pa and *Pv* are the mean arterial and venous pressures, respectively.

Equation (5) is derived by setting the flow due to the mean pressure difference on the arterial side of the microvasculature *(Pa-Pmc)/ra* equal to that on the venous side *(Pmc-Pv)/rv* [29].

Figure 2 shows our estimates of mean capillary pressures as values of ra/rv change in skeletal muscle (mean arterial pressure 95 mmHg and mean venous pressure 5 mmHg). The most striking consequence is the asymmetry between the effects of changes in ra/rv from what is considered to be its usual "control" zone (r_a/r_v equal to 3–4, in skeletal muscle indicated by the shaded rectangle). Small reductions in resistance lead to progressively large increases in *Pmc*, whereas increases in *ra/rv* reduce *Pmc*, but the relative reduction is smaller as r_a/r_v increases.

In the clinic, patients with reduced plasma volume have vasoconstriction in most of the systemic circulation (particularly muscle and skin), increased r_a/r_v, and reduced capillary pressure. Provided the plasma protein concentration and colloid osmotic pressure are not significantly reduced, the balance of effective colloid and hydrostatic pressure differences across the microvascular wall favors fluid movement from the interstitial fluid into the blood. In a healthy blood donor this can result in a return of a limited volume (e.g., 400 mL after a 1 L loss). The process is self-limiting as the protein-free fluid dilutes the plasma protein and vasoconstriction is reduced. The situation is far more complicated with sustained blood loss and with disease or injury that compromise the normal physiological mechanism. Renkin's multiple investigations of the control of microvascular blood flow [25] provide insights into the multiple intrinsic and extrinsic mechanisms that act to regulate local

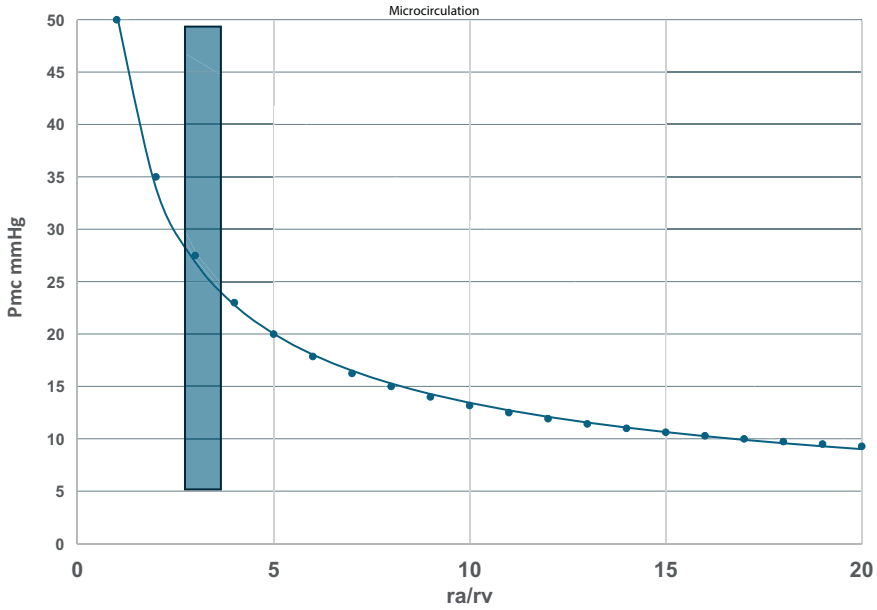

FIGURE 2 | Microvessel pressure as a function of the ratio of resistance to flow on the arterial side of the microcirculation to that venous side (*ra/rv*) as in Equation (5). The shaded rectangle spans the normal range of vascular resistance and capillary pressures. The Figure demonstrates the asymmetry in microvessel pressure changes as the ratio *ra/rv* is modified.

blood flow. These may sustain increased *ra* and reduced microvascular pressure and flow (sympathetic stimulation and high circulating vasoactive agents) or counter the increased *ra* (accumulation of local metabolites and local vasoactive agents) to restore flow but compromise any advantage of reduced capillary pressure. We have outlined a strategy that requires monitoring of plasma oncotic pressure to ensure that the infused volume does not lower plasma oncotic pressure below a threshold where the balance of colloid osmotic pressure in the lung microvessels would result in fluid accumulation in the lung [30].

4 | Convective Versus Diffusive Transport

4.1 | Transport of Plasma Proteins

In his Bowditch lecture, Renkin [5] demonstrated that small pores did not account for the transport of plasma proteins from blood to lymph and instead estimated the permeability-surface area product (*PS*) for a second pathway for plasma proteins larger than albumin. Assuming a steady state between the flux of plasma proteins appearing in the lymph (calculated as lymph flow *L* times lymph protein concentration *CL*) and the transvascular flux of plasma proteins (*Js*), Renkin derived the relation:

$$Js = L \times CL = PS \times (Ca - CL) \tag{6}$$

$$\text{So that } PS = LR/(1 - R) \tag{7}$$

where $R = CL/Ca$. *PS* in Equations (6 and 7) describes mechanisms that depend only on the protein concentration difference between blood and lymph (a dissipative mechanism). To distinguish between diffusion through water-filled pores and transport by vesicles, which were assumed to take up whole packets of plasma, Renkin used plots of the logarithm of mean *PS* values against the logarithm of the molecular weight of the macromolecules. He argued such plots would have a slope of −½ or greater if transport was by diffusion through water filled pores. Because the slope of relations was less than this expected value in several of the microvascular beds he examined, he argued that a mechanism whereby endothelial vesicles shuttled plasma proteins across the microvascular wall independent of their molecular weight was likely to contribute to at least some of the transvascular exchange of larger plasma proteins.

While significant evidence for the uptake and transport of electron dense tracer molecules by vesicles was consistent with their role in transvascular exchange of molecules the size of plasma proteins, theoretical analyses by Perl [31] and new experiments and analyses by Taylor and his associates (Brace et al. [32], Granger and Taylor [33]) demonstrated the need to evaluate the contributions of both dissipative and convective transport to the transvascular exchange of large solutes. They found that, at high lymph flows, the flux of plasma proteins appearing in the lymph could be accounted for by convective transport through large water filled pores in a homoporous membrane model where all the transvascular water flow was through the large pores:

$$Js = L \times CL = L \times (1 - \sigma m) \times Ca \tag{8}$$

here σm is a reflection coefficient that determines the coupling of the protein flux to the flux of water.

Renkin's detailed review [34] extended this approach by accounting separately for convective and diffusive components of plasma proteins through small pores and large pores. He confirmed that, at the high lymph flows, large water-filled pores (characteristic pore radius of 24 nm) carried up to 90% of the transvascular flux of albumin and larger plasma proteins. However, at normal and less elevated pressures, evaluation of the protein flux through separate pathways required far more

detailed analyses. He argued that, because all the water forming the lymph does not flow through large pores, the homoporous analysis likely overestimates their contribution to the plasma to lymph exchange. His new analysis accounted separately for the transport of proteins through small pores and large pores, taking into account the change in the distribution of water flows between these pathways as the microvascular pressure increased and the balance of hydrostatic and effective osmotic pressure in the two pathways changed. Because this was a new and more complex approach, Renkin offered his analysis as a guide to further research rather than reaching definitive conclusions. In this spirit, we first reconstructed his analysis of the convective and diffusive transport of albumin (3.6 molecular radius, a size approaching the small pore radius). These results are discussed below.

The analysis for albumin at a mean microvascular pressure of 16 mmHg (control lymph flows) and more elevated lymph flow (pressure 31.7 mmHg, shaded area) is in Figure 3A. The small pore radius of 4 nm excludes most albumin and restricts diffusion so that the diffusive and convective component of small pore transport is small at both pressures. The hydraulic conductivity (*LpS*) of the large pores is 12% of the hydraulic conductivity of the whole microvascular bed. In large pores, the bulk of the transport is convective (blue columns) and increases as the water flow through the large pore increases at the higher pressure (shaded area). A key result is that, at normal pressures and lymph flow, the total albumin flux attributed to both pore pathways in Figure 3A (0.79 mL/s per 100 g tissue), (0.77 + 0.024) is just over half of the total flux. The estimate at the higher pressure is similar so that, on average at these pressures, 40% of the flux of albumin was not accounted for by porous pathways in skeletal muscle. Renkin attributed this additional flux to vesicle transport. While this estimate was less than his previous value based on Equation (7), Renkin emphasized that the estimates were still tentative. To the extent that more albumin was transported via porous pathways, the contribution of the non-porous pathway would be correspondingly reduced. Thus, the actual contribution of vesicle transport to the total flux across the microvessel wall of plasma proteins remains unresolved. There is a need to further evaluate the contribution of mechanisms involving specific binding sites on the endothelial surface and within caveolae for albumin and albumin bound plasma components [35–37].

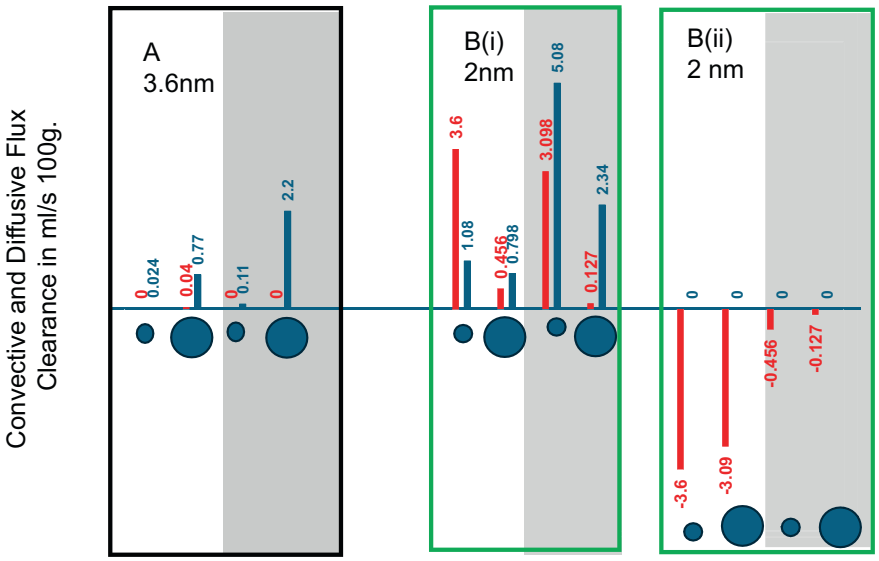

FIGURE 3 | The figure demonstrates the range of microvascular clearance by convective and diffusive transport in skeletal muscle as the solute size and perfusion condition change. Numerical values for each clearance are recorded separately. (A) Illustrates Renkin's estimates of albumin transport through small pores (small, filled circles) and large pore (large, filled circles) at two microvascular pressures, one close to normal (16 mmHg) and an elevated pressure (31.7 mmHg; shaded area). The key observation is that convective transport (blue columns) dominates and most of the convective flux is carried in large pores (0.77 mL/min per 100 g of tissue at the lower pressure and 2.2 mL/min per 100 g at the higher pressure). An additional result from Renkin's analysis is that, at each pressure, the albumin flux by diffusion and convection through porous pathway (both small and large pore) accounts for about 60% of the total flux. Renkin attributed the other 40% of exchange to non-porous, vesicle pathways. The two parts of (B) shows results for a smaller protein (Stokes radius 2 nm), to represent smaller proteins with MW 14-17KDa with the same distribution of water flows though small and large pores as for (A). In (B(i)) protein is circulating in the blood and the key point is that, for this small protein, transport relative to albumin is increased, especially for small pores because of the reduced restriction to transport. However, the relative contributions of diffusion (red columns) and convection (blue columns) differ at each condition. At the lower pressure, there is a close balance of hydrostatic and colloid osmotic pressure in small pores, water flow is relatively low, and diffusion dominates. Water flow increase significantly through small pores as pressure increase (shaded area) and convective transport overtakes diffusion in the small pores. Relative to albumin, transport of the low MW protein is less sensitive to changes in size and pressure in large pores. A striking result is shown in (B(ii)) where the small peptide is in the tissue and diffusion from tissue to blood is in the direction opposite to the water flow. The results are shown for no peptide in the blood. Convective transport of solute occurs only within the pore after the peptide has entered from the tissue. The convection carries some of the peptide in the direction opposite the diffusion gradient, thereby reducing the diffusive flux from tissue to blood (show as net negative transport and expressed as a clearance relative to tissue concentration). At pressure slightly above 31.7 mmHg, this analysis shows that new transport from tissue to blood of the smaller peptide would be zero.

There are also additional sources of uncertainty in these estimates based on lymph collection. The analysis does not take into account the distribution of microvascular pressures within the perfused organ. Also, the assumption that a steady state was established so that the lymph composition reflects only transport across the microvessel wall would be compromised if plasma proteins, present in the interstitial fluid before the experiment began, continued to be washed out into the lymph for longer than expected.

Another area of uncertainty involves the number and distribution of large pores. In Renkin's analysis the number of large was estimated from pore theory assuming the resistance to water within a pore is proportional to the fourth power of the pore radius (Poiseuille's law). Large pores (24 nm radius) occupy only a tiny fraction of the exchange area in the normal microvasculature of skeletal muscle. If distributed uniformly through the microvasculature, only about two large pores would be required in each microvessel (average length of close to 1000 μm). Furthermore, because venular vessels tend to be more leaky than true capillaries, it is likely that these microvessels have a higher frequency of large pores. It follows that there are microvessels (likely arteriolar and true capillaries) with less than 2, and possibly no large pores.

The important conclusion is that individual large pores may be separated by significant distances along individual microvessels. It follows that the plasma protein concentration of the filtrate crossing the capillary wall in regions distant from large pores (several hundred microns) will be lower than in the local region near a large pore. It is not known how such heterogeneity is reflected in the average interstitial fluid composition of plasma proteins. It has been demonstrated that the colloid osmotic pressure difference opposing filtration in most microvascular beds is larger than that expected from the average interstitial protein concentration. The revised Starling Principle accounts for the osmotic pressure opposing filtration, assuming the protein composition of the interstitial fluid close to exchange vessels is less than that of the bulk of interstitial fluid because of filtration through small pores [38].

4.2 | Convection and Diffusion in Small and Large Pores for a Smaller Peptide

We note that while there are many investigations of the transport of larger plasma proteins from plasma to interstitial fluid and lymph, there appear to be few, if any, quantitative studies of the clearance of intermediate sized molecules (MW 10–25 kDa), Stokes radius 1.5–2.5 nm when both diffusion and convection contribute to exchange.

Figure 3B demonstrates how the magnitude of diffusion and convection for macromolecules varies with molecular size and the magnitude and direction of transvascular diffusion relative to transvascular water flow. Figure 3Bi shows the distribution of protein flux between large and small pores for a 2 nm protein when the distribution of water flows between large pores and small pores is the same as used for the albumin in Figure 3A. As expected, transport in small pores is significantly increased relative to albumin because restriction to diffusion is decreased and the coupling of solute to water flow (measured as 1-σ) increased. Diffusion and convection both contribute to exchange through pores but the relative contribution of diffusion relative to convection is different in small pores versus large pores and at the two pressures examined. Experiments that enable some of these predictions to be tested are described in Figure 5 below [39, 40].

Figure 3Bi demonstrates that, despite its smaller molecular radius, convective transport from blood to tissue dominates in the large pores. This is an important result because it shows that convective transport dominates exchange whenever the selectivity of the microvessel wall is compromised by disease and the injured microvessel wall has the equivalent of more large pores. Furthermore, under such inflammatory conditions, any tendency to increase the hydrostatic pressure difference across the large pores further increases convective transport and delivers plasma protein into the interstitial space at concentrations approaching those in the plasma. On the other hand, strategies to modulate the transvascular hydrostatic pressure difference and to exploit the transient formation of large pores to deliver high molecular weight therapeutic agents in, for example, cancer therapy have been based on this principle [41–43].

A novel interaction of diffusion and convection is illustrated in Figure 3Bii. We evaluated the extent to which water flows (normal blood to tissue direction) oppose diffusion of the small peptides from tissue to blood. As far as we can determine, this condition has not been described in detail. Here we show the condition where there is initially no small peptide in the plasma, so there is no convective transport into the pores from the blood side. Importantly, the extent to which solute that diffuses into the pore from the tissue to reach the plasma is significantly reduced by convective transport within the pore. Depending on the water velocity and the coupling of solute to the water flow within the pore, a fraction of the solute flux due to diffusion into the pore from the tissue will be carried back into the tissue, and the net transport from tissue to blood will be reduced. Thus, net transport from tissue to blood occurs only when diffusion exceeds this convective transport capacity within the pore. At a pressure slightly above the value of 31.7 mmHg, net exchange from tissue into blood by direct diffusion across the microvessel wall would be zero. This type of analysis can enable further investigations of the relative importance of local diffusion from tissue to blood versus transport via lymph to the distribution of inflammatory cytokines produced by cells in the tissue. Examples include TNF-α (MW 17 kDa) produced under ischemia and interleukin-1 (17.5 kDa) after exposure to lipopolysaccharide [44]. Other possible applications include the transdermal introduction of therapeutic peptides where the agent first accumulates in the tissue, or the local distribution of peptide toxins after a snake bite, for example.

5 | Experiments in Individually Perfused Microvessels

5.1 | Osmotic Transient Experiments

In 1972, Renkin joined the Michel lab in Oxford, UK, for a short sabbatical. While Renkin was in the lab, Curry also joined as a postdoctoral fellow. Renkin wanted to evaluate the

ways that the methods to cannulate and perfuse individual microvessels being developed in the Michel Lab might provide additional ways to investigate microvascular exchange. The first collaborative project based on the use of the Landis Micro-Occlusion Method to measure transvascular water flows [45] demonstrated that osmotic flows across the capillary due to concentration differences of small solutes (sodium chloride, glucose, sucrose) were independent of molecular size, indicating that the osmotic flows resulting from a small solute concentration difference across the microvessel wall were mainly across an exclusive water pathway in parallel with the porous pathways for hydrophilic solutes [46]. On the basis of this result, Renkin and Curry [47] re-analyzed the osmotic transient experiments from Renkin's thesis. They found that a similar exclusive water pathway (likely aquaporin pathways [48]), accounting for 20%–50% of the total vessel wall hydraulic conductivity, must be present in the microvessels of cat and dog hindlimb used in the original experiments. This meant that the key assumption in the original analysis was not compromised by any failure to account for osmotic flow through the pores themselves [49].

Two further examples of results from individually perfused vessels that address questions posed by Renkin's research are described briefly below.

5.2 | Flow Dependent Permeability in Small Solutes

Figure 4 demonstrates the flow dependent permeability coefficients of sodium fluorescein in an individually perfused microvessel of rat mesentery [50]. All the perfusion conditions (perfusate composition, flow velocity, surface area, time to respond, and capillary pressure) were directly measured. The key observation in Figure 4A is that permeability increased linearly with increased perfusion velocity. Such a mechanism would act to match local exchange capacity (*PS*) to local blood flow, thereby reducing the effects of heterogeneity on exchange. Figure 4B demonstrates that, in the same experiment, there was a rapid adjustment in permeability as flow rate is increased and decreased. The flow dependent response was abolished when the vessels were exposed to nitric oxide synthase inhibitors. The flow dependence of the permeability coefficient was ignored in the development of Equation (3) to describe solute flux as flow increased in a vessel. It is not known if such flow regulated permeability occurs in all organs, and its role in different organs remains to be determined.

5.3 | Macromolecule Transport in Individually Perfused Microvessels

Figure 5 shows investigations in individual microvessels that address Renkin's long-term interest in the relation between microvascular permeability and the morphology of the microvascular wall [51]. The figure combines results from an early phase of long-term investigations in the Michel and Curry labs inspired by Renkin's encouragement to investigate the ultrastructure of the wall in microvessels where permeability was directly measured. We use Figure 5 here to point to further experiments to

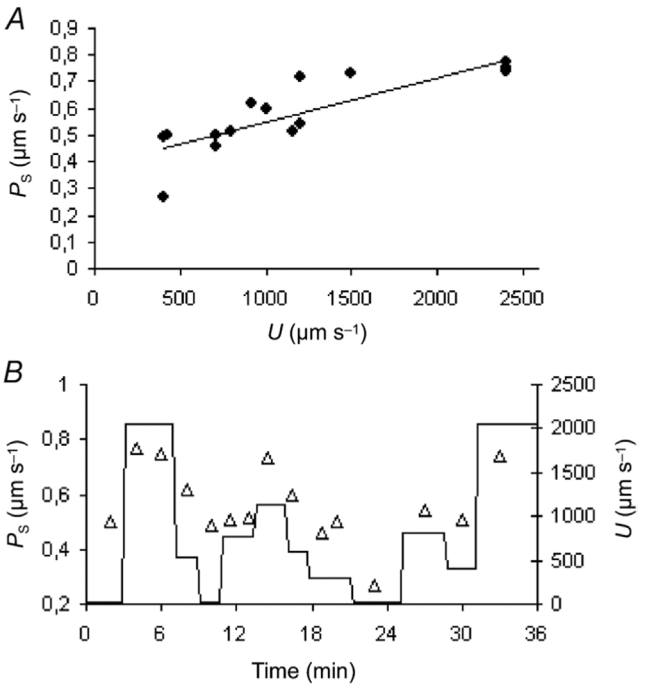

FIGURE 4 | Measurement of the permeability of the small test solute sodium fluorescein (MW 576) as the perfusate flow is changed over a physiological range in an individually perfused microvessel of rat mesentery. Part A is the linear relation between the magnitude of the permeability coefficient and the perfusate velocity. Part B show details of the same experiment as the time course of changes in the perfusion velocity (right axis) shown by the connected solid lines and the corresponding values of permeability (triangles) measured within 2–4 min of the change in perfusion velocity (left axis). Such flow dependent permeability may be one of the mechanisms to heterogeneity of exchange in a microvascular bed by matching exchange capacity to blood flow. Reproduced with permission from 48.

determine the physical nature of small pores and large pores, the role of the glycocalyx as a key determinant of the size of the small pores, and the role of plasma constituents to maintain normal permeability.

The left panel shows measurements of the clearance of the intermediate sized protein alpha-lactalbumin (MW 14KD, Stokes radius 2 nm) as successive components of plasma are removed from the microvessel perfusate to increase permeability [39, 40]. The right panel shows changes in the microvessel glycocalyx under the same experimental conditions as in the left panel [52]. In particular, the extent to which the glycocalyx extends into the vessel lumen from the endothelial cell membrane is measured by labeling the upper surface of the glycocalyx exposed to the perfusate with the electron dense tracer cationic ferritin. More recent investigations in rats in which the glycocalyx is labeled using a fluorescently labeled antibody to the glycocalyx component heparan sulfate show a similar pattern of glycocalyx change [53]. The key result is that in the vessels with the lowest permeability state (lowest curve), the glycocalyx extends more than 100 nm into the lumen. Also, the slope of the relation between clearance pressure is quite flat, and the relative contributions of diffusive and convective flux as pressure increases are close to those expected for transport through small pores as in Figure 3Bi.

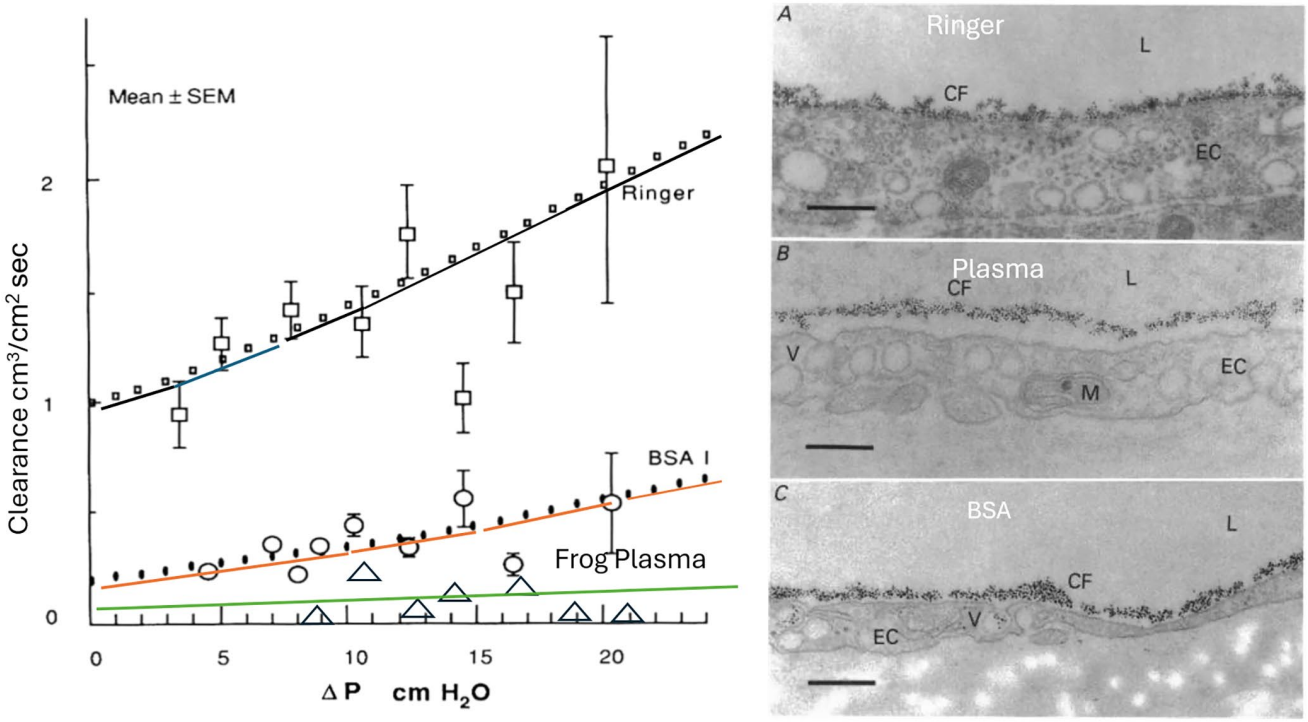

FIGURE 5 | In the left panel the mean values of the clearance of alpha-lactalbumin measured in individually perfused microvessels are shown as a function of the pressure in microvessel as the composition of the perfusate is changed from plasma to albumin (BSA) and Ringer alone. There is an increase the permeability and decrease selectivity of the microvessel wall with these changes in perfusate composition. The intercept of the ordinate is an estimate of the solute permeability coefficient in the absence of transvascular fluid flow. The slope of the relation between clearance and pressure is a measure of the rate the convective component of exchange increases with capillary pressure. In the right panel images of the outer surface of the endothelial glycocalyx labeled with cationic ferritin in vessels perfused under the same conditions in the left panel as those in which the clearances of alpha-lactalbumin were measured. The outer surface of the endothelial glycocalyx is labeled with the electron dense marker to indicate the extent that the glycocalyx extends into the microvessel lumen. Part A is modified from the originals [39, 40] with permission. Part B modified from 52 with permission.

In contrast are the conditions where all plasma components are removed and the perfusate is Ringer alone (top curve). Permeability is increased and the glycocalyx is absent or collapsed onto the endothelial surface. The slope of the plot of clearance versus pressure is steep and convective transport dominates because the selectivity of the barrier is decreased and more transvascular water flow occurs through large pores. Transport is dominated by larger pores, as shown in Figure 3Bi. These and similar observations formed part of the experimental support for the hypothesis that the endothelial glycocalyx formed at least part of the size-limiting structure at the capillary wall that was characterized in terms of the restriction to diffusion through small pores. A detailed model has been developed to account for the flow of water and plasma solutes crossing the microvessel wall through an ordered glycocalyx at the luminal entrance to the junctions between endothelial cells [54]. According to this fiber matrix-junction break model of capillary permeability, spacing between the fibers and bound proteins in the glycocalyx determines the size of the small pores, while the number of pores is determined by the size and frequency of breaks in the continuity of the strands of molecular complexes between adjacent endothelial cells that normally seal the intercellular junctions. Large pores are formed when the glycocalyx barrier is removed and molecules can penetrate into the junctions. Further evaluation of this model requires new information on the composition of the glycocalyx, especially in the region of the intercellular junctions, the size and order of spaces within the glycocalyx, and the role of charge and chemical interactions between the glycocalyx and plasma constituents. The role of the glycocalyx as the primary molecular sieve in fenestrated capillaries, including the renal glomerulus, is also an area of research [55, 56].

Finally, we note that the actions of plasma constituents such as plasma and albumin to modify permeability, as in Figure 5, address Renkin's suggestion that sustained increases in microvascular permeability after exposure to inflammatory agents involve more than transient gap formation between endothelial cells [51]. Recent investigations demonstrate that albumin and red cells regulate the availability of the plasma glycolipid sphingosine −1-phosphate (S1P) to the capillary wall [53, 57, 58]. Binding of S1P to an endothelial receptor regulates the release of matrix metalloproteins that degrade the glycocalyx. S1P also regulates multiple mechanisms to stabilize the cortical actin networks of endothelial cells that determine endothelial cell shape, cell–cell, and cell matrix adhesion. Much remains to be understood about these and similar mechanisms that maintain normal permeability and restore the microvascular barrier after exposure to inflammatory conditions.

6 | Perspectives

We have reviewed Renkin's use of simplified uniform models of microvascular exchange units to describe the fundamental functions of the microcirculation: a cylindrical pore to characterize the barriers to exchange of water and solutes, a cylindrical capillary to distinguish flow-limited exchange from diffusion-limited exchange between blood and tissue, and a membrane with large and small pores to determine the relative contributions of diffusion and convection to macromolecule exchange between blood and lymph. The key idea linking his fundamental observations with the function of intact microvessels is that local blood flow, microvascular pressure, and permeability of the vascular wall are not uniform within microvascular beds. While some heterogeneity is the result of the normal physiological mechanisms controlling flow and pressure, and permeability, there is growing awareness that disease increases heterogeneity, leading to significantly compromised microvascular function. Examples from recent literature (since 2020) include microvascular dysfunction in cardiovascular and renal disease [59, 60], septic shock [61] and diabetes and other metabolic disturbances [62, 63]. Models of networks with a range of microvascular properties [64, 65] provide theoretical investigations with the aim of understanding the patterns of response associated with different forms of heterogeneity (disturbed local flow, loss of capillaries) and thereby guide clinical care, as suggested by McClatchey et al. earlier [27, 28].

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Data Availability Statement

Data supporting this study are included within the article.

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