Microvascular blood viscosity in vivo and the endothelial surface layer

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Pries, A. R., and T. W. Secomb. Microvascular blood viscosity in vivo and the endothelial surface layer. Am J Physiol Heart Circ Physiol 289: H2657–H2664, 2005. First published July 22, 2005; doi:10.1152/ajpheart.00297.2005.—The apparent viscosity of blood in glass tubes declines with decreasing diameter (Fåhraeus-Lindqvist effect) and exhibits a distinctive minimum at 6–7 μm. However, flow resistance in vivo in small vessels is substantially higher than predicted by in vitro viscosity data. The presence of a thick endothelial surface layer (ESL) has been proposed as the primary cause for this discrepancy. Here, a physical model is proposed for microvascular flow resistance as a function of vessel diameter and hematocrit in vivo; it combines in vitro blood viscosity with effects of a diameter-dependent ESL. The model was developed on the basis of flow distributions observed in three microvascular networks in the rat mesentery with 392, 546, and 383 vessel segments, for which vessel diameters, network architecture, flow velocity, and hematocrit were determined by intravital microscopy. A previously described hemodynamic simulation was used to predict the distributions of flow and hematocrit from the assumed model for effective blood viscosity. The dependence of ESL thickness on vessel diameter was estimated by minimizing deviations of predicted values for velocities, flow directions, and hematocrits from measured data. Optimal results were obtained with a layer thickness of ~0.8–1 μm for 10- to 40-μm-diameter vessels and declined strongly for smaller diameters, with an additional hematocrit-dependent impact on flow resistance exhibiting a maximum for ~10-μm-diameter vessels. These results show that flow resistance in vivo can be explained by in vitro blood viscosity and the presence of an ESL and indicate the theoretically effective thickness of the ESL in microvessels.

flow resistance; hemodynamics; hematocrit; glyocalyx; microcirculation; red blood cell

AFTER ABOUT 75 years, the investigation of blood rheology in glass tubes of small luminal diameters, triggered by the initial reports of Martini et al. (19) and Fåhraeus and Lindqvist (8), has resulted in a relatively consistent set of experimental data (1, 8, 12, 19, 20, 27, 36, 46; Barbee JH and Cokelet GR, personal communication). These data have been used to derive a parametric empirical description of the dependence of apparent blood viscosity on tube diameter and hematocrit (27). The most impressive property of this relation is the reduction of viscosity for red blood cell suspensions (e.g., with a flow fraction of red blood cells or discharge hematocrit of 40%) relative to the viscosity of the suspending medium alone from ~3.2 in tubes with diameters above ~1 mm to only ~1.25 in tubes with diameters of ~6–7 μm, the so-called Fåhraeus-Lindqvist effect.

However, there are also indications that the direct extrapolation of such data to the situation in living microvessels in vivo may not be adequate. Among those concerns were the effects of irregular vessel diameters (28, 38), the effects of vascular bifurcations and relatively short vascular segments, and the presence of leukocytes (9, 47). In classical experiments using a double-micropipette technique, Lipowsky et al. (16, 17) showed that the apparent blood viscosity is much higher in relatively straight, unbranched microvessels than in glass tubes with corresponding inner diameters. A study based on the analysis of overall pressure drop in microvascular networks (32) suggested that only about one-third of the observed pressure drop could be explained on the basis of blood viscosity as measured in vitro. On the basis of the comparison of the measured flow distribution with predictions of a mathematical flow simulation, the authors derived a viscosity relation that exhibited a substantially attenuated Fåhraeus-Lindqvist effect and much higher effective viscosities in smaller vessels. However, this relation was not based on a physical model for the causes of the increased effective viscosity.

In the last decade, the experimental evidence for the presence of a comparatively thick layer on the endothelial surface [endothelial surface layer (ESL)] has expanded substantially (30). In addition to indirect evidence from measurements of hematocrit (6, 15) and flow resistance (32, 33), the layer and its modification by physiological and pathophysiological stimuli have been visualized (49, 50). A number of theoretical approaches have been proposed to analyze the mechanical properties of the ESL and its impact on blood rheology and exchange between the blood and the tissue (4, 39–42, 45, 52).

The aim of the present study was to investigate 1) whether the distribution of flow resistance in microvessels in vivo can be explained by a simple physical model combining the well-established in vitro viscosity relation with the assumption of an ESL and 2) what effective thickness the layer would then exhibit in vessels of different sizes.

MATERIALS AND METHODS

Experimental data. The animal preparation, the intravital microscopic setup, the scanning procedures, and the methods for optical determination of hematocrit and flow velocity have been described in detail elsewhere (23, 31). After approval from the university and state authorities for animal welfare, male Wistar rats were prepared for intravital microscopy. Catheters were inserted into the jugular vein and carotid artery to allow the control of anesthetic level and fluid balance by continuous intravenous infusion of physiological saline (24 ml·kg⁻¹·h⁻¹) containing pentobarbital sodium (0.3 mg/ml) and by monitoring of heart rate and arterial pressure. The small bowel was exteriorized, and fat-free portions of the mesentery were selected for investigation. Papaverine (10⁻⁴ M) was continuously applied to suppress active vessel tone.

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The area of tissue supplied by a selected microvascular network was scanned with an immersion objective (model 25 × 0.6 NA SW, Leitz) and recorded on videotape using monochromatic illumination (448-nm wavelength) for offline densitometric measurement of hematocrit (flow fraction of red blood cells; discharge hematocrit (H_D)) (25). A second scan was performed using asynchronous strobe illumination (model 11360-1, Chadwick-Helmuth, El Monte, CA) to allow measurement of flow velocity. The scanning procedures lasted ~30 min. A digital image analysis system was used to measure hematocrit and flow velocities offline from the video recordings (23). Spatial correlation analysis of recordings obtained with asynchronous illumination allowed measurement of velocities along the centerline of a microvessel up to ~40 mm/s (24). Values were averaged over ~4 s to obtain mean velocity data independent of pulsatile fluctuations. From repeated measurements of flow velocity and hematocrit in 190 segments in one of the networks, conservative estimates of relative root mean square velocity error of 0.25 and absolute root mean square hematocrit error of 0.11 were obtained (32). These errors represent the precision, but not the accuracy, of the methods used. Although it is very difficult to assess the accuracy of the in vivo measurements, estimates based on mass balance considerations have been published elsewhere (3).

During the scanning procedure, photographs were taken from which we assembled a photo montage of the network, which was used to measure the length of all segments. In the mesenteric preparations, all vessels were located in one plane, so it was not necessary to apply correction for viewing angles. Anatomic vessel segment diameters were determined from the photo negatives. Three networks with 392, 546, and 383 vessel segments supplying ~30- to 50-mm² areas with overall blood flow rates of 750, 402, and 224 nl/min, respectively, were recorded and analyzed.

Simulation of network hemodynamics. The mathematical simulation used to estimate the distribution of blood flow with the microvascular networks has been described in detail elsewhere (32). A system of linear equations obtained by imposing conservation of flow at each node (segment junction) in the network was used to assess the pressures at each node given the hematocrit (according to phase separation), the effective viscosity (according to the Fåhraeus-Lindqvist effect), and the effective diameter for each segment (linear analysis, inner loop). The nodal pressures were then used to update the volume flow rate, hematocrit, and viscosity in each segment (rheological analysis, outer loop), and the procedure was repeated. The respective loops were iterated until a satisfactory degree of convergence was achieved. Inputs for the model calculation are the experimentally recorded network structure (segment connection matrix, segment length, and diameter) and network boundary conditions. The boundary conditions comprise the volume flow rate, the hematocrit in the feeding segments of the network, and volume flow rates in all draining segments. In addition to the network data, the model calculations are based on parametric descriptions of rheological phenomena as follows.

Phase separation effect. The distribution of red blood cells and plasma at microvascular bifurcations was represented according to the approach described earlier (31) with modifications to render predictions more robust for extreme combinations of input hematocrit and vessel diameter. The fractional flow of erythrocytes into one daughter branch (FQ_d) is calculated from the respective fractional blood flow FQ_b as follows:

\[ \log_{10} FQ_d = A + B \log_{10} \left[ \frac{FQ_b - X_0}{1 - 2X_0} \right] \] (1)

where \( \log_{10} x = \ln x / (1 - x) \) and the parameters A, B, and X_0 defining the phase separation characteristics of the bifurcation were obtained from linear fits to experimental data obtained in the rat mesentery (26).

\[ A = -13.9 \left( \frac{D_b^2/D_d^2 - 1}{(D_b^2/D_d^2 + 1)(1 - H_b)/D_b} \right) \] (2)

\[ B = 1 + 6.98(1 - H_b)/D_b \] (3)

\[ X_0 = 0.964(1 - H_b)/D_b \] (4)

where \( D_b, D_d, \) and \( D_e \) are the diameters of the daughter branches and the mother vessel and \( H_b \) is the discharge hematocrit in the mother vessel (i.e., the flow fraction of red blood cells in the blood passing through the vessel). In these relations and throughout, all diameters are given in micrometers.

Effective blood viscosity (Fåhraeus-Lindqvist effect). The viscosity estimation in the model simulation was based on the results of a meta-analysis of experimental data from numerous in vitro studies of red cell suspensions perfused through ~1-mm- down to 3.3-μm-diameter glass tubes (27). These data were used to derive a parametric description of relative apparent viscosity for suspensions of human erythrocytes as a function of tube diameter and hematocrit (“in vitro viscosity law”) according to the following equation:

\[ \eta_\text{app} = 1 + (\eta_{0.45} - 1) \left( \frac{1 - H_b}{1 - 0.45} \right)^{0.45} \] (5)

The relative apparent blood viscosity for a fixed \( H_b \) of 0.45 (or 45%), \( \eta_{0.45} \), is given by

\[ \eta_{0.45} = 220 \exp(-1.3D) + 3.2 - 2.44 \exp(-0.66D) \] (6)

where \( D \) is the lumen diameter of the tube.

The shape of the viscosity dependence on hematocrit is governed by the factor C, according to

\[ C = (0.8 + e^{-0.078D}) \left( -1 + \frac{1}{1 + 10^{-11} \cdot D^2} \right) + \frac{1}{1 + 10^{-11} \cdot D^2} \] (7)

which represents a sharp transition from a linear to a convex dependence of viscosity on hematocrit between 7 and 9 μm, respectively. Consistent with the experimental data, these equations describe a substantial decline of apparent viscosity with decreasing diameter < 1 mm. In the diameter range 5–7 μm, a minimum of the relative apparent viscosity is obtained, and the presence of suspended red blood cells only slightly increases flow resistance in glass tubes.

ESL thickness and effective viscosity. Data on the thickness of the ESL and its dependence on vessel diameter are sparse (18, 30, 35, 44, 48, 50). Therefore, a number of possible functions were tested for the dependence of layer thickness on \( D \), the apparent anatomic vessel diameter as measured by intravital microscopy. The data set used in the present study contains few >40-μm-diameter vessels. An ESL thickness of 2.6 μm in 150-μm rat mesenteric small arteries was reported by van Haaren et al. (48). The layer thickness functions were therefore chosen to asymptote to a maximal thickness (W_max) of 2.6 μm at large vessel diameters. The alternative functions were evaluated on the basis of the deviations of measured flow velocities, flow directions, and hematocrits from the respective values predicted by the hemodynamic model simulation.

Of the functional forms considered for layer thickness as a function of diameter, the best results were obtained by considering a family of functions consisting of a sum of two components: 1) a monotonically increasing function (W_a) with a hyperbolic asymptote to the maximal thickness (W_max) at large diameters and 2) a biphasic function (W_peak) growing linearly to a peak at a critical diameter (D_crit) and then decaying exponentially to zero at large diameters.

\[ W_a = \begin{cases} \frac{D - D_{\text{off}}}{D + D_{\text{off}} - 2D_{\text{off}}} W_{\text{max}} & \text{if } D < D_{\text{off}} \\ 0 & \text{if } D \geq D_{\text{off}} \end{cases} \] (8)

\[ W_{\text{peak}} = \begin{cases} 0 & \text{if } D < D_{\text{off}} \\ E_{\text{amp}} e^{-(D - D_{\text{crit}})/D_{\text{amp}}} - D_{\text{off}} & \text{if } D_{\text{off}} \leq D < D_{\text{crit}} \\ E_{\text{amp}} e^{-(D - D_{\text{crit}})/D_{\text{amp}}} & \text{if } D \geq D_{\text{crit}} \end{cases} \] (9)

Both functions approach zero at a lower threshold diameter (D_off), below which the layer thickness is zero. A sharp corner in the variation of layer thickness with diameter as predicted by Eq. 9 is likely not present in nature. However, when a term was added that allowed smoothing of the corner to a variable degree, the best results
in the optimization procedure (see below) were obtained with the parameter controlling the degree of smoothing set to zero. The assumed physical thickness of the layer \((W_{ph})\) was calculated by adding the asymptotic component and the peak component multiplied by a variable parameter \((E_{peak})\)

\[ W_{ph} = W_{as} + W_{peak} E_{peak} \]  \( (10) \)

During the optimization process, a better fit to observed network flow behavior was obtained if the peak component of the thickness function was assumed to have an additional hematocrit-dependent effect on flow resistance. This was implemented by calculating the effective thickness of the layer \((W_{eff})\) according to

\[ W_{eff} = W_{as} + W_{peak} [1 + H_{2}, E_{fit}] \]  \( (11) \)

where \(E_{HD}\) gives its sensitivity to \(H_{2}\).

In the simulations, the effective viscosity in each segment of the network is given by \(\eta_{\text{eff}} = \eta_{\text{vivo}} \cdot (D/D_{\text{eff}})^{4}\), where \(D_{\text{eff}}\) is the effective diameter, which takes into account the influence of the ESL (with the assumption of no or very little flow through the ESL); i.e., \(D_{\text{eff}} = D - 2W_{ph}\). The corresponding in vitro viscosity \(\eta_{\text{vivo}}\) is calculated using the relations defined by Eqs. 5–7, but with the measured anatomic diameter \(D\) replaced by \(D_{ph} = D - 2W_{ph}\), which defines the part of the vessel cross section that is available for free flow. The flow resistance in each segment is then computed using Poiseuille’s law, based on \(\eta_{\text{vivo}}\) and \(D\).

**Optimization procedure.** The free parameters of Eqs. 8–11, \(D_{off}, D_{crit}, D_{so}, E_{amp}, E_{width}, E_{peak},\) and \(E_{HD}\), were estimated by minimizing the mean square deviation between measured flow velocities and flow velocities obtained from the model simulation \(V_{\text{ERR}}\). For the calculation of \(V_{\text{ERR}}\), values of red blood cell velocity \(\langle V_{c}\rangle\) were used. In the presence of an ESL, the relation between mean blood velocity \(\langle V_{b}\rangle\) calculated for the anatomic vessel diameter) and \(\langle V_{c}\rangle\) varies strongly with the assumed thickness of the layer. However, the experimental measurements of flow velocity (32) are based on the motion of red blood cells, and, after correction for the spatial averaging effect (22), correspond to \(\langle V_{c}\rangle\). Thus this value allows a comparison of measured and predicted velocities that is independent of assumptions on effective layer thickness.

Predicted values of \(\langle V_{c}\rangle\) were derived from the calculated volume flow rate \(Q\) in each vessel. A previously published parametric description of the Fähraeus effect for blood flow in vitro (31) was used

\[ \langle H_{c}/H_{b}\rangle_{\text{vivo}} = \langle H_{b}\rangle + (1 - \langle H_{b}\rangle)(1 + 1.7 e^{-0.45 D} - 0.6 e^{-0.011 D}) \]  \( (12) \)

where \(\langle H_{T}\rangle\) is tube hematocrit. The corresponding in vivo Fähraeus effect in the presence of the ESL was calculated by replacing \(D\) with \(D_{ph}\) in Eq. 12 and multiplying by a factor to reflect the difference between the anatomic diameter and the diameter available for flow

\[ \langle H_{c}/H_{b}\rangle_{\text{vivo}} = \langle H_{b}/H_{2}\rangle_{\text{vivo}} \cdot (D_{ph}/D_{as})^{2} \]  \( (13) \)

Cell velocity was then calculated as follows

\[ \langle V_{c}\rangle = \langle V_{b}\rangle \langle H_{c}/H_{b}\rangle_{\text{vivo}} \quad \text{where} \quad \langle V_{b}\rangle = Q/(\pi D_{as}^{2}/4) \]  \( (14) \)

The parameter optimization by minimizing \(V_{\text{ERR}}\) was performed using the multidimensional downhill simplex approach (21), in which the parameter values are successively adjusted in multiple model runs. In addition to \(V_{\text{ERR}}\) the mean square deviation of discharge hematocrits \(H_{\text{ERR}}\) and the number of segments in which the measured and predicted flow directions did not coincide \((N_{\text{INV}})\) were determined to assess the quality of the obtained results.

**RESULTS**

Figure 1 shows the overall deviations of hemodynamic parameters obtained from the flow simulation from the corresponding values measured during intravital microscopy of mesenteric microvascular networks for several different assumed thicknesses with respect to the dependence of apparent viscosity on diameter and hematocrit. Switching from the in vitro viscosity law to a previously published in vivo viscosity relation (32) led to a significant reduction in the velocity and hematocrit deviations \((V_{\text{ERR}}\) and \(H_{\text{ERR}}\) as well as the number of segments in which the predicted flow direction did not coincide with the measured flow direction \((N_{\text{INV}})\).

Further reductions in \(V_{\text{ERR}}\) and \(N_{\text{INV}}\) were obtained if the present ESL-based in vivo viscosity relation described in Eqs. 8–11 was used. The optimized parameter values for a retardation of flow due to the layer that is independent of hematocrit \((E_{HD} = 0)\) were \(D_{off} = 2.4 \mu m, D_{crit} = 10.5 \mu m, D_{so} = 100 \mu m, E_{amp} = 1.23, E_{width} = 0.03,\) and \(E_{peak} = 0.6,\) However, \(H_{\text{ERR}}\) values were not reduced relative to the in vitro or the previously published in vivo relations when a hematocrit-independent effect of the ESL was assumed. The lowest values in all three deviation parameters, including \(H_{\text{ERR}},\) were obtained with the ESL-based viscosity relation, including a hematocrit-dependent impact of the layer on flow resistance \((E_{HD} > 0)\) and the optimized parameter values \(D_{off} = 2.4 \mu m, D_{crit} = 10.5 \mu m, D_{so} = 100 \mu m, E_{amp} = 1.1, E_{width} = 0.03,\) and \(E_{peak} = 0.6,\) and \(E_{HD} = 1.18.\) The deviation levels obtained are in the range of the theoretical minimum that would be expected on the basis of known experimental measurement errors in the determination of vessel diameters and flow velocities (32). The mean absolute deviation between measured velocities and the results of the flow simulation was 0.54 ± 0.79 (SD) mm/s and showed no dependence on vessel diameter.

The dependence of ESL thickness on diameter as predicted by the optimized parameter values listed above is shown in Fig. 2. For any vessel that allows passage of red blood cells, it can be assumed that a maximal layer thickness corresponds to the vessel diameter reduced by the minimal diameter of a maximally elongated red blood cell (2.8 \(\mu m\)) divided by 2. The range of values that do not comply with this condition is shown in Fig. 2 (dark shaded area). The assumptions on effective layer thickness that resulted in the best match between experimental data and predictions of the hemodynamic model are given for different levels of \(H_{D}\). They exhibit a marked peak at a vessel diameter just above 10 \(\mu m\) (10.5 \(\mu m\)), the height of which increases with increasing \(H_{D}\).

In interpreting this result, it must be acknowledged that the values in Fig. 2 may not directly represent the morphological thickness of the ESL. In contrast, they correspond to the reduction in effective vessel diameter that is necessary to explain the observed flow resistance in microvessels of a given apparent vessel diameter at a given \(H_{D}\). Thus, for a given morphological thickness of the layer, its impact on flow resistance may grow with an increase in red blood cell interaction with the layer. Such an increased interaction is enforced in vessels with diameters above the range where red blood cells can only travel one after the other (single-file flow), i.e., above \(~6 \mu m\). At low hematocrits, red blood cells can travel in such vessels without interfering with the vessel wall or the ESL (Fig. 3). With increasing hematocrit, the probability increases that more than one red blood cell is found in a given cross section through the vessel. This leads to cell-to-cell interactions, which effectively displace cells in a radial direction toward the wall, where they may experience retardation because of the presence of the ESL. This effect seems to be strongest in the range where two or three cells interact with
each other on one cross section of the vessel, i.e., for diameters ~10 μm.

The diameter- and hematocrit-dependent increase of effective layer thickness shown in Fig. 2 may thus represent an increased hemodynamically relevant interaction of red blood cells with the layer. It is therefore likely that the morphological layer thickness corresponds to the boundary of the vertically hatched area (corresponding to \( W_{ph} \)) in Fig. 2. This indicates a more-or-less constant layer thickness down to ~10 μm. The sharp decrease below this level results from the resistance of red blood cells to deformation, diminishing layer thickness with reduction of the vessel diameter to maintain the part of the vessel diameter available for red blood cells.

A number of alternative versions for Eqs. 9 and 10, which predict different diameter dependence of the effective layer, were tested. Among these were a sigmoidal increase of layer thickness with diameter with and without an overshoot in a given diameter range and modifications of the present equations allowing for a smooth, rounded transition between the two slopes. However, these alternatives consistently resulted in higher levels for the deviation parameters \( V_{ERR} \), \( H_{ERR} \), and \( N_{INV} \).

Figure 4 shows values for effective blood viscosity relative to plasma viscosity. By multiplying by plasma viscosity (~1.05 cP for rat blood at 37°C), relative blood viscosity can be converted to absolute viscosity and used to estimate volume flow for a given pressure drop and vessel diameter as measured by light microscopy (without visualization of the ESL). For

![Fig. 1](image1)
![Fig. 2](image2)

Fig. 1. Mean square deviation of measured flow velocities \( (V_{ERR}) \), number of segments with inverted flow directions \( (N_{INV}) \), and mean square deviation of hematocrits \( (H_{ERR}) \) based on comparisons of model simulation results with observed values. Averages with standard errors are given for 3 microvascular networks in the mesentery. In vitro 92, simulations using viscosity relation based on data obtained in glass tubes (27); in vivo 94, simulations using relation derived previously for microvascular networks (32); in vivo ESL, simulations using relations obtained in the present study based on the assumption of an endothelial surface layer (ESL) with a diameter-dependent thickness. For the latter, 2 alternatives, with \( (H_D \) dependence: yes) and without \( (H_D \) dependence: no) a hematocrit-dependent effect of the ESL, are shown.
The Faåhræus effect results from the difference in flow velocity between red blood cells and plasma. It may be challenged whether the region within the ESL should be included in the intravascular volume when HT is computed, as done here. However, in most intravital investigations, it is not possible to distinguish between the ESL and the free-flowing plasma. The anatomic vessel diameter is therefore used to calculate H_T (6, 15). Also, the components of the ESL are in dynamic exchange with the free-flowing plasma, and there is some hydraulic flow through the layer (4, 34, 40, 41), rendering the distinction between plasma and ESL material dependent on the time scale that is applied.

**DISCUSSION**

The present study shows that the estimated variation of flow resistance in microvessels in vivo with anatomic diameter and with hematocrit may be explained by the viscosity relations as determined in vitro if the presence of an ESL is taken into account. To account for this variation, we assumed that the effective thickness of the layer varies with vessel diameter and, less importantly, with hematocrit. The approach used to obtain these results is based on the comparison of flow and hematocrit distributions in microvascular networks with predictions of a mathematical flow simulation using the established in vitro viscosity relations and several assumptions on effective layer thickness. This approach circumvents the experimental problems of direct viscosity measurements in single microvessels in vivo (16, 17) by analyzing distributions in a large number of vessels within a network. This approach, however, also suffers from measurement errors during the experimental determination of vessel diameters and lengths as well as flow velocities (3, 32). Also, 90% of the vessels included in the analysis exhibit diameters between -5 and 40 μm. Thus data presented for larger microvessels reflect the extrapolation to data for small arteries by van Haaren et al. (48).

The dependence of effective viscosity on diameter shown in Fig. 4 may appear surprising, with effective viscosity decreasing as diameter decreases below ~10 μm. However, it should be remembered that flow resistance is proportional to effective viscosity divided by the fourth power of diameter. Flow resistance continues to increase with decreasing diameter in this range. According to the present results, the rate of increase would, however, be reduced because of the progressive thinning of the ESL for <10-μm-diameter vessels.

A limitation of approaches in which the effective thickness of the ESL is inferred from an observed increase in flow resistance is the inability to distinguish between variations of the structural thickness of the layer and the interactions that flowing red blood cells may have with the layer. Such a differentiation requires the use of direct visualization, as shown, for example, by Vink and Duling (50, 51). On the basis of the available information, it is not likely that the structural layer thickness shows a distinctive peak at ~10 μm. Furthermore, there are no conceivable physical explanations for an increase in ESL thickness with increasing hematocrit. Thus it appears likely that the structural thickness of the wall layer exhibits the simpler dependence on diameter shown by the vertically hatched area in Fig. 2. According to this assumption, the diameter- and hematocrit-dependent increase in the hemodynamic effect of the layer corresponds to specific flow patterns in the relevant diameter range of vessels.

A major characteristic of blood rheology (2, 11, 29) is the particulate nature of the red blood cells. This imposes a specific length scale in the micrometer range on rheological phenomena as related to vessel diameters. Up to ~6 μm, the vessel cross...
section can accommodate only one red blood cell, and single-file flow conditions prevail, at least for hematocrit below ~60%. Under these conditions, red blood cells deform and exhibit shapes that allow them to travel in the more central flow regions (10, 37, 43), limiting their interaction with the vessel wall or the ESL. An increase in hematocrit leads to the alignment of more cells along a given length of the vessel, but not to a substantial widening of the red blood cell column. For example, two cases illustrated in Fig. 3 (HD 15, D 7 μm and HD 30) correspond to a linear increase in viscosity with increasing hematocrit, as reported for small tubes in experimental studies (27).

Starting at very high hematocrit levels in 6-μm tubes or lower levels in somewhat larger tubes, more than one cell must be squeezed into a given vascular cross section, resulting in multifile flow. For the 7-μm tube in Fig. 3, the transition to multifile flow conditions begins at a hematocrit of ~45%. For a >9-μm-diameter tube, this process starts at low hematocrits, and the relation between viscosity and hematocrit is consistently nonlinear (27). In the in vitro viscosity relation, this is reflected by the variation of the shape factor C, which according to Eq. 7 generates a transition from a linear to a convex relation between viscosity and hematocrit for tube diameters increasing from 7 to 9 μm. If the ESL thickness of ~0.5–0.7 μm is considered, this transition would be seen in vivo in a range of anatomic vessel diameters of 8–10.4 μm. This corresponds closely to the peak in the effective layer thickness (Fig. 2). The transition to multifile flow generates more cell-to-cell interactions, which are accentuated by the fact that cells travel on different streamlines and, thus, may exhibit different average velocities (13, 14). Cell-to-cell interactions and collisions lead to a radial displacement of cells toward the wall, bringing them into close contact with the ESL. This diameter- and hematocrit-dependent mechanism is thought to be the cause of the observed increase in effective layer thickness centered at ~10 μm.

In previous studies (31), several potential mechanisms were proposed for the discrepancy between experimental estimates of apparent viscosity in microvessels in vitro and in vivo. In the present work, we have shown that the discrepancy can be fully accounted for by the effects of an ESL with diameter-dependent thickness, which exerts additional hematocrit-dependent restriction on flow for ~10-μm diameters, coupled with a relation between apparent viscosity, diameter, and hematocrit established on the basis of in vitro observations.

The range of ESL thickness inferred by this approach is in general agreement with values estimated by other techniques. On the basis of direct micropipette measurements, Lipowsky...
and co-workers (16) reported mean blood viscosity levels of 4.2 cP in microvessels with mean diameters of ~30 µm. The discrepancy between their values and in vitro values (~1.5 cP) can be accounted for by assuming a wall layer thickness of ~3.4 µm. To reconcile the hematocrit measurements by Duling and Desjardins (7) and Klitzman and Duling (15) in capillaries with the law of mass conservation, a layer on the order of 1–1.2 µm would be required. From a meta-analysis of hemodilution studies, Pries et al. (34) inferred a layer thickness of ~1.5 µm. Using a double-indicator-dilution technique, Rehm et al. (35) estimated the total ESL volume in patients to be ~720 ml. With the assumption of an endothelial surface of ~350 m² (30), the corresponding average ESL thickness would be 2 µm. Vink and co-workers developed the dye-exclusion technique to visualize the ESL thickness directly and reported values of ~0.4–0.5 µm in capillaries (50) and up to 2.6 µm in small arteries (48). Using microparticle image velocimetry, Long et al. (18) and Smith et al. (44) estimated values of ~0.5 µm for ~25- and 45-µm-diameter venules. One possible cause of the apparent discrepancy between the lower values reported from dye exclusion and microparticle image velocimetry approaches in true microvessels and the higher values from studies focusing on flow resistance may be related to the difference between the effective physical thickness of the layer and its effect on red blood cell flow. Although the data of van Haaren et al. (48) indicate an increase of ESL thickness toward larger microvessels, experimental support for the decline of the ESL thickness below ~10 µm is not available.

The present results do not rule out the possibility that other effects, such as flow disturbances due to irregularity of vascular lumens, bifurcations, and leukocytes, contribute to increased resistance in vivo but are consistent with the hypothesis that the ESL is the main cause of the discrepancy. Independent of the underlying physical mechanisms, these results provide an improved representation of the hemodynamics of microvascular networks that can be used in simulations of microvascular flow.

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