Microviscometry reveals reduced blood viscosity and altered shear rate and shear stress profiles in microvessels after hemodilution

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Communicated by William R. Schowalter, Princeton University, Princeton, NJ, April 27, 2004 (received for review July 20, 2003)

We show that many salient hemodynamic flow properties, which have been difficult or impossible to assess in microvessels in vivo, can be estimated by using microviscometry and fluorescent microparticle image velocimetry in microvessels >20 μm in diameter. Radial distributions in blood viscosity, shear stress, and shear rate are obtained and used to predict axial pressure gradient, apparent viscosity, and endothelial-cell surface-layer thickness in vivo. Based solely on microparticle image velocimetry data, which are readily obtainable during the course of most intravital microscopy protocols from systemically injected particle tracers, we show that the microviscometric method consistently predicted a reduction in local and apparent blood viscosity after isovolemic hemodilution. Among its clinical applications, hemodilution is a procedure that is used to treat various pathologies that require reduction in peripheral vascular-flow resistance. Our results are directly relevant in this context because they suggest that the fractional decrease in systemic hematocrit is >25–35% greater than the accompanying fractional decrease in microvascular-flow resistance in vivo. In terms of its fundamental usefulness, the microviscometric method provides a comprehensive quantitative analysis of microvascular hemodynamics that has applications in broad areas of medicine and physiology and is particularly relevant to quantitative studies of angiogenesis, tumor growth, leukocyte adhesion, vascular-flow resistance, tissue perfusion, and endothelial-cell mechanotransduction.

Ating back to the work of Fåhræus over 70 years ago, studies related to blood flow in the microcirculation (1–4) have been featured prominently in scientific investigations across various fields, including endothelial-cell mechanotransduction, inflammation, vascular permeability, angiogenesis, and tissue engineering. Nevertheless, no method has been developed for either quantitatively predicting or measuring the salient dynamic, kinematic, and rheological properties of microvascular blood flow in vivo other than in the single-file flow regime within capillaries of 5–8 μm in diameter (4–7). All attempts at analyzing microvascular blood-flow properties in microvessels above the capillary range have depended on knowledge of quantities, such as the axial pressure gradient within the vessel and the red-cell concentration of blood discharged by the vessel, which are essentially unknown in these vessels (2, 3, 8, 9). In the absence of any satisfactory way of estimating these hemodynamic quantities, researchers have had to resort to indirect methods, which often contain errors of nearly an order of magnitude.

In 1830, the physiologist J. L. M. Poiseuille arrived at his celebrated law relating the volumetric flow rate of a Newtonian fluid in a cylindrical tube to the difference in pressure acting across the length of the tube (10). A century later, Fåhræus and Lindqvist showed that, because of the phase separation between red cells and plasma that occurs in microvessels and glass capillary tubes (11), an increasing departure from Poiseuille’s law is observed with decreasing diameter (12). However, although they were able to establish this fact, which has come to be known as the Fåhræus–Lindqvist effect, the means for predicting blood-flow parameters on theoretical grounds in either glass capillary tubes or microvessels beyond the single-file flow regime eluded scientists working in this area for the remainder of the century.

Because red blood cells are less concentrated near the wall than near the center of microvessels, mean red-cell velocity exceeds mean plasma velocity. This disparity in mean velocities gives rise to the so-called Fåhræus effect, which is associated with a decrease in the instantaneous volume fraction of red cells in the vessel or tube hematocrit, Hr, relative to the red-cell concentration discharged from the vessel, or discharge hematocrit, HD. In general, neither Hr nor HD are equal to the systemic hematocrit, Hsys, which is obtained from a large artery or vein, because red cells and plasma distribute unevenly at microvascular bifurcations (13). Measurements of Hr in microvessels in vivo have been attempted either by using microphotometric methods (8, 14) or by counting labeled red cells (15), neither of which are reliable in microvessels more than ~20 μm in diameter. Measurements of Hr have been attempted in microvessels in vivo either by micropipette aspiration and centrifugation (16), which is extremely cumbersome and feasible only in some tissues, or by a microphotometric method (13), which assumes the same Fåhræus effect in glass tubes and microvessels of the same size. The accuracy of this assumption, however, is dubious in light of recent evidence of the influence of the endothelial surface layer (ESL) on plasma flow near the wall of microvessels in vivo (17, 18). In addition to the difficulties associated with determining Hr and HD in vivo, attempts (8, 19) at measuring or estimating either axial pressure gradient or flow resistance accurately in microvessels have been unsuccessful in vivo.

The central tenet of our approach (20), which we hereafter shall refer to as the microviscometric method, is that distributions in the local viscosity, μ(r), as a function of the radial position, r, over the cross section of glass capillary tubes and microvessels, can be determined analytically from the cross-sectional axial velocity distribution, vr(r), where vr(r) can be extracted from particle tracers in the flow by using intravital fluorescent microparticle image velocimetry (μ-PIV) (17, 21, 22). In addition to the viscosity distribution, μ(r), we can use the microviscometric method to predict quantitatively various flow parameters in microvessels in vivo, including axial pressure gradient, dp/dz; volume flow rate, Q; and the relative apparent blood viscosity, ηrel, defined as the ratio of steady volume–flow rates per unit pressure drop of blood plasma relative to whole blood. The velocity distribution, vr(r), is related kinematically to the shear rate distribution, γ(r) = dvz/dr, where γ(r) is assumed...
to be related constitutively to the shear stress distribution, \( \tau(r) = \mu(r) \gamma(r) \), for a linearly viscous fluid. By means of rigorous analysis of glass-tube experiments \textit{in vitro}, where pressure gradient and feed hematocrit can be measured directly, and isovolumic hemodilution experiments \textit{in vivo}, where the change in systemic hematocrit is known, we provide quantitative validation of the microviscometric method and show a decrease in local and apparent blood viscosity in individual microvessels as a direct result of reducing systemic hematocrit.

**Materials and Methods**

**Analytical Methods.** The analysis (20) on which the microviscometric method is based invokes the continuum approximation and regards the heterogeneous red-cell suspension as a homogeneous, continuously varying, and linearly viscous incompressible fluid that has a spatially nonuniform viscosity distribution (23) over the vessel cross section. Cokelet (2) found support for the continuum approximation in his studies by using physiological concentrations of red blood cells suspended in plasma flowing at physiological shear rates in glass tubes as small as 20 \( \mu \text{m} \) in diameter. As for our constitutive assumption, our results are consistent with the linearly viscous approximation because, as we will show, over most of the tube or vessel cross section, shear rates are more than \( \approx 50 \text{s}^{-1} \) under physiologically typical flow rates. Blood viscosity at a given hematocrit is nearly constant at such shear rates (24). Thus, we assume that at shear rates of more than \( \approx 50 \text{s}^{-1} \), blood is Newtonian in the sense that local viscosity depends only on local thermodynamic state and local hematocrit and is independent of shear rate.

It is further assumed that, in postcapillary venules, the flow is steady and the velocity profile is axisymmetric and fully developed. A brief summary of the analysis of an axisymmetric, fully developed, and steady incompressible flow of a linearly viscous fluid having a spatially varying viscosity distribution over the cross section of both cylindrical glass tubes and ESL-lined microvessels is provided in Supporting Text, which is published as supporting information on the PNAS web site. Full details are given in ref. 20.

**Glass-Tube Experiments.** Fluorescent \( \mu \)-PIV (21, 22) was performed by using a method described in ref. 17. Fluoresbrite yellow–green microspheres (0.47 \pm 0.01 \( \mu \text{m} \), 1.05 \text{g/cm}^3; Polysciences) were visualized by using stroboscopic double-flash (5- to 16.67-ms apart; Stroboskop 11360, Chadwick-Helmuth, El Monte, CA) epi-illumination, and recordings were made by using a VE-1000CD charge-coupled device camera (Dage–MTI, Michigan City, IN) on an S-VHS recorder (Panasonic, Secaucus, NJ).

The \textit{in vitro} perfusion system consisted of a 3-ml feed reservoir and a horizontally mounted glass capillary tube (length, 27.5, 26.2, and 20 mm; i.d., 54.2, 50.7, and 81.0 \( \mu \text{m} \), respectively), which were connected by a microhematocrit tube (i.d., 1.2 mm) and silastic tubing (Dow-Corning), to a 10-ml downstream reservoir that could be manipulated vertically with a Vernier tee (protocol no. 2474). All mice appeared to be healthy and were 8–14 weeks of age. Microscopic observations of microspheres \textit{in vivo} were made in venules of the exteriorized cremaster muscle and followed the \textit{in vitro} protocols described above and in Supporting Text. No optical correction of the radial positions of microspheres measured \textit{in vivo} was necessary because the difference in the refractive indices of blood plasma and the surrounding tissue is negligibly small (17).

**Hemodilution.** Mice were given a 1.0-ml i.p. injection of physiological saline to help prevent fluid imbalance before cremaster exteriorization. During hemodilution, the carotid artery cannula was allowed to bleed into an inverted syringe tube for accurate volume measurements. Saline was infused through the jugular vein cannula at a volume flow rate matched to the carotid bleed rate until 0.75 ml was exchanged. Blood samples (30 \( \mu \text{l} \)) were drawn from the carotid artery at least 25 min before and after hemodilution for systemic hematocrit measurements. Up to two vessels per animal were recorded before and after hemodilution and analyzed offline by using the microviscometric method, as described above and in Supporting Text. Exact vessel locations were noted by using muscle striations as markers.

**Data Analysis.** Video recordings were digitized with PREMIERE software (Adobe Systems, Mountain View, CA; final resolution 5.43 pixels per \( \mu \text{m} \)) and then analyzed with the public domain National Institutes of Health IMAGE program (available at http://rsb.info.nih.gov/nih-image), as described in refs. 17 and 26. The flash-time interval for \( \mu \)-PIV recordings was chosen such that the two images for a given microsphere were 3- to 30-\( \mu \text{m} \) apart. The center-to-center distance between these two images and the shortest distance between the microsphere center and the vessel or tube wall were measured for \( \approx 75 \) microspheres in each glass capillary tube and \approx 50 microspheres in each microvessel. Measurements were restricted to a section of capillary tube or microvessel \( < 180 \mu \text{m} \) or 15 \( \mu \text{m} \) in axial length, respectively. Each \( \mu \)-PIV data set was used to extract an axisymmetric velocity profile, \( v_z(r) \), by following the methods described in Supporting Text.

**Results**

To test the validity of the microviscometric method in a model system, we obtained fluorescent \( \mu \)-PIV data over the cross section of glass capillary tubes (i.d., \approx 50–80 \( \mu \text{m} \)) that were perfused steadily with saline, plasma, and red-cell suspensions in plasma, as described above. Distributions predicted in glass capillary tubes by using the microviscometric method were qualitatively similar to those shown in Fig. 1 (see Figs. 5–14, which are published as supporting information on the PNAS web site). For red-cell suspensions, results consistently revealed a concentrated red-cell core and a cell-poor region near the vessel wall (see Figs. 5–12). Furthermore, the shear-rate distributions consistently showed a nearly linear variation over \( \approx 50–70\% \) of the tube cross section around the center of the tube and a highly nonlinear variation near the tube wall (see Figs. 1 and 5–12). The nearly parabolic velocity distributions and linear shear rate distributions predicted in saline–perfused glass tubes (see Figs. 1 A and C, 13, and 14) after optically correcting the measured radial position of each microsphere provide confidence in the optical correction procedure that we used for all of our glass-tube \( \mu \)-PIV data. Further validation is provided in ref. 17.

Evidence to support the validity of the microviscometric method \textit{in vitro} is shown in Fig. 2, in which directly measured
values of \( \frac{dp}{dz} \) and empirically estimated (3) values of \( \eta_{rel} \) agree closely with their corresponding values predicted by using the microviscometric method for each \( \mu \)-PIV data set. Furthermore, these results provide support for the validity of using the continuum approximation of blood to obtain estimates of \( \frac{dp}{dz} \) and \( \eta_{rel} \) in glass capillary tubes \( \approx 50 \, \mu \text{m} \) in diameter.

To apply the microviscometric method to blood flow in microvessels \( \textit{in vivo} \), a generalization is introduced (20) to account for the hemodynamic influence of the ESL (17, 18, 27, 28). Expressions for \( \gamma(r) \) and \( \frac{dp}{dz} \) apply in microvessels (see Supporting Text) if the tube radius, \( R \), is replaced by \( a \), where \( a \) is the radial location of the effective hydrodynamic interface between the blood in the lumen and the ESL (17, 20). It is assumed that red cells and particle tracers do not invade the ESL (7, 17, 28–30) and that plasma flow through the ESL can be well approximated with the Brinkman equation (17, 20, 29, 31, 32), where the hydraulic resistivity, \( K \), of the ESL is taken to be more than \( \approx 10^9 \, \text{dyns/cm}^4 \) (1 dyn = 10 \( \mu \text{N} \)) (7, 17, 29, 30). The thickness, \( R - a \), of the ESL is estimated by following the methods described in ref. 20, in which the value of \( a \) is determined by minimizing the least-squares error in the fit to the \( \mu \)-PIV data. The minimum least-squares error for the 12 microvessels that we analyzed (i.d., 34.2 \( \pm \) 1.7 \( \mu \text{m} \)) occurred over ESL thicknesses ranging 0.29–0.71 \( \mu \text{m} \), with an average thick-
the local volume flow rate integrated over the vessel cross section. If both \( \eta_{\text{rel}}(r) \) and \( \mu(r) \) are positive functions over the vessel cross section and decrease monotonically with increasing radial position, it will always be the case that \( \mu_1/\mu_2 < 1 \), just as the Fåhraeus effect implies that \( H_T/\bar{H}_D < 1 \). The relative apparent viscosity, \( \eta_{\text{rel}} \), however, is a measure of flow resistance of whole blood relative to blood plasma.

If the species-specific transport relationship, \( H(\mu) \), were available for mouse blood, the distribution \( \mu(r) / \mu_2 \) could be replaced by \( H(\mu(r)) \) in Eq. 1 to provide expressions for \( H_T \) and \( H_D \) (see Supporting Text). In the absence of such data, we cannot estimate \( H_T \) or \( H_D \) directly; however, we can nevertheless use Eq. 1 to quantitatively evaluate the accuracy of the microviscometric method in vivo by noting that the percentage of decrease in \( \mu_D \) would likely be very similar to the percentage of decrease in its counterpart, \( H_D \) (and likewise for \( \mu_T \) and its counterpart, \( H_T \)), because the transport relationship enters into the integrand of each term in the numerator of the percentage of decrease in \( H_D \) in the same way as it does in the denominator. That is, we assume that \( [(\mu_D) - (\mu_D)] / (\mu_D) \approx [(H_D) - (H_D)] / (H_D) \), where \( i \) and \( f \) refer a particular quantity to its value in the same vessel before and after systemic hemodilution, respectively.

To test the validity of this assumption quantitatively, we again turn to the results of our glass-tube experiments in which \( H_D \) is known. We can regard any pair of glass-tube experiments having different measured values of \( H_D \) as an “in vitro” hemodilution experiment, in which the higher and lower values of \( H_D \) in the pair can be thought of as corresponding to before and after hemodilution, respectively. Substituting the distribution \( \mu(r) \) predicted from the microviscometric method into Eq. 1, we have determined \( \mu_1 \) and \( \mu_2 \) for each of our glass-tube experiments involving red-cell suspensions in plasma. For any two glass-tube experiments having different measured values of \( H_D \), the accuracy with which we can predict the percentage of difference in the directly measured values of \( H_D \) from the percentage of difference in our predicted values of \( \mu_2 \) is shown in Fig. 4. It is evident from these results that, even without knowledge of the specific transport relationship, \( H(\mu) \), we can indirectly infer the percentage of difference in \( H_D \) from our predictions of the percentage of difference in \( \mu_2 \) with an accuracy that is similar to that which was achieved in our direct predictions of the rheological quantities shown in Fig. 2.

Having established, in glass tubes, the accuracy with which the percentage of change in \( \mu_D \) can be used to infer the percentage of change in \( H_D \), provides some measure of confidence for using this metric in vivo. Further support, however, can be found directly from our in vivo hemodilution experiments (see Figs. 3 and 27–31) by comparing the average percentage of decrease in \( \mu_D \) with the average percentage of decrease in \( H_D \) after isovolemic hemodilution (see Table 2). In all five vessels analyzed in vivo, \( \mu_T \) and \( \mu_D \) decreased after systemic hemodilution.

For an average decrease in \( H_{\text{sys}} \) of 33.5 ± 1.0\%, the average percentage of decrease in \( \mu_T \) and \( \mu_D \) (and, by inference, \( H_T \) and \( H_D \)) across all five vessels was predicted to be 33.5 ± 5.2 and 36.3 ± 4.8\%, respectively. Because red-cell screening and plasma skimming at vessel branch points gives rise to network heterogeneousity in the discharge hematocrits of individual microvessels, the percentage of decrease in \( H_D \) for any individual microvessel is not, in general, equal to the percentage of decrease in the systemic hematocrit of the animal. Although it was impractical to measure the percentage of decrease in \( H_D \) directly after hemodilution in each of the vessels that we analyzed, there is evidence that in microvessels more than 20 \( \mu \)m in diameter, the average discharge hematocrit across \( N \) microvessels in a network does indeed approach the systemic hematocrit with increasing \( N \) (13). Hence, the mean fractional decrease in \( \mu_D \) should approach the mean fractional decrease in \( H_{\text{sys}} \) as the number of analyzed

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**Fig. 2.** Predictability of \( dp/\text{dz} \) and \( \eta_{\text{rel}} \) by using microviscometric analysis of \( \mu \)-PV data obtained from glass capillary tubes in vitro. Ratio of the measured to predicted value of \( dp/\text{dz} \) versus the corresponding measured value (A) and ratio of the empirically estimated (3) to predicted value of \( \eta_{\text{rel}} \) versus the corresponding empirically estimated value (B). Predicted values were determined by applying the microviscometric method to the \( \mu \)-PV data obtained from the glass-tube experiments. The light and dark shaded regions span, respectively, one and two standard deviations in the distributions around unity. The standard deviation corresponds to 23\% for \( dp/\text{dz} \) and 16\% for \( \eta_{\text{rel}} \). The correlation coefficient, \( r_c \), is shown for its corresponding predicted quantity.

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**Fig. 3.** As an example, Fig. 3 shows the results of one in vivo hemodilution experiment in a mouse cremaster-muscle venule (diameter, \( \sim 40 \mu \)) in diameter). Results for other hemodilution experiments are shown in Figs. 27–31 and Table 2, which are published as supporting information on the PNAS web site. To facilitate a quantitative comparison between the results of our hemodilution experiments and quantities that can be predicted by the microviscometric method, we define \( \mu_T \), which is analogous to tube hematocrit, \( H_T \), and \( \mu_D \), which is analogous to discharge hematocrit, \( H_D \), where

\[
\mu_T := \frac{1}{A} \int_a \frac{\mu(r)}{\mu_0} \frac{\text{d}A}{\mu_0}, \quad \mu_D := \frac{1}{Q} \int_a \frac{\mu(r)}{\mu_0} v_T(r) \text{d}A, \tag{1}
\]

\( A \) is the cross-sectional area of the vessel lumen, \( Q \) is the volume flow rate in the vessel, and \( \mu_0 = \mu(a) \). The analogous quantities, \( H_T \) and \( H_D \), correspond to the mean instantaneous red-cell concentration in the vessel and the mean red-cell flux fraction through the vessel, respectively (see Supporting Text). It is evident from Eq. 1 that \( \mu_T \) corresponds to the mean instantaneous normalized viscosity over the vessel cross section, whereas for a unit volume flow rate, \( \mu_D \) is simply the product of the local viscosity (which depends on the local red-cell concentration) and
vessels increases. This trend is observed in the in vivo results presented here.

As with \(\mu_D^I\) and \(\mu_D^D\), our microviscometric analysis predicted that in every vessel that we evaluated, flow resistance, as measured by the relative apparent viscosity, \(\eta_{rel}\), was also seen to decrease (25.1 \pm 6.1\%), on average) after systemic hemodilution.

Collectively, these results provide the first direct and quantitative estimate of the accompanying fractional decrease in local and apparent blood viscosity in individual microvessels that is associated with the clinically relevant procedure of isovolemic hemodilution.

A noteworthy trend observed in these results is that the average percentage of decrease in measured \(H_{sys}\) and predicted \(\mu_D^D\) were, respectively, 33% and 45% greater than the average percentage of decrease in predicted \(\eta_{rel}\) in vivo. This trend was observed also in our glass-tube studies, in which the average percentage of decrease in measured \(H_D\) and predicted \(\mu_D^D\) were, respectively, 26% and 48% greater than the average percentage of decrease in predicted \(\eta_{rel}\). This trend has potentially important clinical implications in the context of hemodilution procedures because it is the decrease in \(\eta_{rel}\), and not the decrease in \(H_D\) or \(H_{sys}\), that quantitatively determines the decrease in microvascular-flow resistance. Thus, these results suggest that the fractional decrease in systemic hematocrit is \(\approx 25-35\%\) greater than the accompanying fractional decrease in microvascular-flow resistance.

**Discussion**

By using fluorescently labeled platelets as endogenous particle tracers, previous work has revealed blunted velocity profiles in microvessels in vivo and showed that the Poiseuille flow approximation underestimates wall shear rate in these microvessels (21,
flow rates, the interfacial shear rate, shown, under physiologically typical discharge hematocrits and no-slip condition at the vessel wall. However, as these results estimate wall shear rate. Most studies make this estimate by technique, which uses mean blood-flow velocity, derived from microviscometric method.

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the vessel wall causes fluid shear stress and fluid shear rate at the luminal endothelial-cell surface [i.e., wall shear rate, \( \gamma(R) \), and wall shear stress, \( \tau(R) \)] to be very nearly zero for values of hydraulic resistivity more than \( \approx 10^9 \) dynes/cm² (17). Consequently, these results show that the appropriate quantitative metrics characterizing near-wall microfluidics in microvessels are the interfacial shear rate, \( \gamma(a) \), and interfacial shear stress, \( \tau(a) = \mu_a \dot{\gamma}(a) \), which can now be predicted by using the microviscometric method.

The most popular and widespread method for estimating microvascular blood-flow parameters in vivo is the dual-slit technique, which uses mean blood-flow velocity, derived from centerline velocity measured by cross correlation (8, 33), to estimate wall shear rate. Most studies make this estimate by assuming a Poiseuille flow in the microvessel and imposing the no-slip condition at the vessel wall. However, as these results show, under physiologically typical discharge hematocrits and flow rates, the interfacial shear rate, \( \gamma(a) \), corresponding to the shear rate at the effective interface between the ESL and the free lumen, is, on average, about five times greater than estimates of wall shear rate based on the dual-slit technique assuming Poiseuille flow (see Table 1).

Micro rheological phenomena in terminal vascular beds impact broad areas of medicine and physiology. We have demonstrat ed that the microviscometric method allows estimation of the axial pressure gradient and relative apparent viscosity of steady flows in microvessels 20–50 \( \mu \)m in diameter, without the need to impale these vessels with micropipettes and without any prior assumptions about the Fähraeus or Fähraeus–Lindqvist effects in vivo. Because the experimental methods used to obtain \( \mu \)-PIV data are compatible with most intravital microscopy protocols, the micro viscometric method can provide a system atic, standardized approach by which microvascular-blood-param eters can be estimated in vivo. The radial distributions in viscosity, shear stress, and shear rate predicted here, as well as the means for determining them, will allow detailed quantitative modeling of the hemodynamics of microvascular networks in vivo. Furthermore, the ability of the microviscometric method to detect the presence of the ESL and estimate its hydrodynamically relevant thickness in microvessels \( >20 \mu \)m in diameter is essential to the quantitative aspects of a broad range of fields in microvascular physiology. In particular, by using the microvisc ometric method before and after various treatments to degrade the ESL (17, 18, 20, 34), we are now poised to gain insight into the role of the ESL in inflammation, endothelial-cell mechanotransduction, microvascular hemodynamics, and flow-mediated mechanisms in angiogenesis (27). Finally, by using the microviscometric method before and after isovolumic hemodilution, we have directly demonstrated the impact of this procedure on hemodynamics in individual microvessels. These results have direct clinical relevance because systemic hemodilution has been used to save blood during surgery and to reduce peripheral resistance, and it is sometimes used in the treatments of Me nieres disease, polycythemia vera, sickle-cell anemia, and newborns with a systemic hematocrit in excess of \( \approx 70\% \). Because data similar to the data underlying the present analysis can be obtained in many organs and tissues, including those that are not transparent and require fluorescent epi-illumination, it is likely that data sets with broad applicability to physiology and pathophysiology will now become available.

We thank A. L. Butterworth for assistance in data acquisition from video tape. This work was supported by Whitaker Foundation Grant TF-02-0024, National Science Foundation Grant BES-0093985 (to E.R.D.), and National Institutes of Health Grants HL64381 and T32GM 08715-01A1 (to K.L.).

Summary of Analysis for the Microviscometric Method in Glass Tubes. Here, we summarize the essential analytical elements of the microviscometric method in glass tubes. Full details are given in ref. 1.

In all of our glass tube studies, the microparticle image velocimetry ($\mu$-PIV) data were obtained $\approx 1.5$ cm from the inlet of the glass tube. With a typical Reynolds number on the order of $10^{-2}$ for these experiments, it is reasonable to assume that entrance effects are negligible. We, therefore, assume the flow to be fully developed and axisymmetric. In terms of the axial velocity component, $v_z$, and pressure, $p$, the momentum equation for an axisymmetric, fully developed, steady incompressible flow of a spatially varying, linearly viscous fluid in a cylindrical tube of radius $R$ is given in cylindrical coordinates ($r, \theta, z$) by

$$
\frac{1}{r} \frac{d}{dr} \left( \mu(r) r \frac{dv_z}{dr} \right) = \frac{dp}{dz}, \quad 0 < r < R,
$$

where it can be shown (1) that the axial pressure gradient,

$$
\frac{dp}{dz} = \frac{2 \mu_R}{R} \left. \frac{dv_z}{dr} \right|_{r=R},
$$

and the local normalized dynamic viscosity of blood is given by

$$
\frac{\mu(r)}{\mu_R} = \frac{r}{R} \left. \frac{(dv_z/dr)|_{r=R}}{dv_z/dr} \right|_{r=R} = \frac{r}{R} \frac{\dot{\gamma}(R)}{\dot{\gamma}(r)}, \quad 0 < r < R.
$$

Here, the viscosity evaluated at the tube wall, $\mu_R = \mu(R)$, is determined by minimizing the least-squares error in the difference from unity of the ratio of the measured to predicted values of $dp/dz$ for all of the in vitro data sets analyzed, excluding the saline-perfused tubes. From this calibration we estimate $\mu_R$ as being very nearly equal to that of plasma viscosity, $\mu_p$, obtained from a capillary viscometer ($\mu_R \simeq 0.95 \mu_p$, where $\mu_p$ was found to have an average value of $1.20 \pm 0.07$ cP at $23^\circ$C).

In terms of the viscosity distribution, the relative apparent viscosity, $\eta_{rel}$, defined as the ratio of steady volume-flow rates per unit pressure drop of blood plasma relative to whole blood, is given by

$$
\eta_{rel} = \left( 8 \int_0^1 \int_{r/R}^1 \frac{\sigma d\sigma}{\mu(\sigma)/\mu_R} \tilde{r} d\tilde{r} \right)^{-1}.
$$
The tube hematocrit, defined as the mean instantaneous red-cell concentration in the vessel, and the discharge hematocrit, defined as the mean red-cell flux fraction through the vessel, are given, respectively, by

\[ H_T := \frac{1}{A} \int_A H(r) \, dA \quad \text{and} \quad H_D := \frac{1}{Q} \int_A H(r) \, v_z(r) \, dA, \tag{5} \]

where \( A \) is the cross-sectional area of the tube lumen, \( Q \) is the volume flow rate in the tube, and \( H(r) \) is the local hematocrit derived from Eq. 3 and a species-specific transport relationship, \( H(\mu) \), obtained from rotational viscometric data (2). The hematocrit distribution, \( H(r) \), reflects the time-averaged hematocrit at any point in the tube cross section, and as such, it is predicted to vanish only at the tube wall. Even in an approximately steady flow, experimental observations have shown (3–6) that red cells transiently invade the plasma-rich region of microvessels, and contribute to the local hematocrit and viscosity there. Thus, in a time-averaged sense, the local hematocrit is considered to be nonvanishing throughout the vessel lumen.

In principle, the tube and discharge hematocrits are derivable from the viscosity distribution given by Eq. 3 if \( H(\mu) \) were known for a particular species. However, even without \( H(\mu) \), one can nevertheless estimate the fractional change in \( H_T \) and \( H_D \) by considering the fractional change in \( \mu^*_T \) and \( \mu^*_D \), which we define in glass tubes as

\[ \mu^*_T := \frac{1}{A} \int_A \mu(r) \, dA \quad \text{and} \quad \mu^*_D := \frac{1}{Q} \int_A \mu(r) \, \mu_r \, v_z \, dA. \tag{6} \]

It is evident from Eq. 6 that \( \mu^*_T \) corresponds to the mean instantaneous normalized viscosity over the vessel cross section, and is therefore analogous to \( H_T \). Likewise, \( \mu^*_D \) is analogous to \( H_D \). Just as the local temperature is determined by the energy content in a volume of fluid (and can be used to predict energy flux if the material-specific equation of state for the heat capacity is known), so too is the local value of \( \mu(r) \) determined by the number of red cells in a volume of blood [and can be used to predict red-cell flux if the species-specific transport relationship, \( H(\mu) \), is known]. As such, \( \mu^*_D \) is an indicator of the red-cell flux fraction through the vessel.

**Summary of Analysis for the Microviscometric Method in Microvessels.** In microvessels, we again assume the flow to be axisymmetric and fully developed, the latter being reasonable *in vivo* for profiles
measured more than one vessel diameter away from bifurcations (7,8). In independent studies using endogenous particle tracers (5, 8), a parameter used to quantitatively determine asymmetry in the velocity profile was not significantly different from zero, suggesting that velocity profiles were nearly axisymmetric in most microvessels. Other groups have reported significantly asymmetric red-cell profiles in venules of the rabbit omentum (9) and hamster retractor muscle (10). However, in both of these studies, velocity distributions were determined from a small number of endogenous red-cell tracers that are likely to be too large to provide the necessary spatial resolution required to draw conclusions about symmetry. It has also been reported that red blood cells exhibit erratic deviations in radial position and velocity during transit through 400-μm-long sections of venules in rat spinotrapezius muscle (6). Despite these deviations, erythrocytes from daughter branches of a confluent venular bifurcation remained separated for up to 250 μm downstream of the bifurcation (11). However, redistribution of plasma over the vessel cross-section would likely lead to axisymmetric velocity profiles even in these cases. The fluorescent microspheres used in the present study, being much smaller and more regularly shaped than endogenous particle tracers, provided very high spatial resolution for the velocity profiles we were able to extract. In most of the vessels that we examined, distributions appeared nearly axisymmetric.

In order to extend the microviscometric method to blood flow in microvessels in vivo, a generalization is introduced (1) to account for the hemodynamic influence of the endothelial surface layer (ESL) on the luminal vessel wall (12–14). In particular, we identify two distinct regions, which we refer to as the free lumen, where 0 ≤ r ≤ a, and the annular porous layer (corresponding to the ESL), where a ≤ r ≤ R. Here, a is the radial location of the effective hydrodynamic interface between blood in the free lumen and the ESL (14). In terms of the axial velocity component, v_z, in the free lumen, and pressure, p, the momentum equation in the free lumen for axisymmetric, fully developed, steady incompressible flow of a linearly viscous fluid having radially varying viscosity, μ(r), is given by Eq. 1 if the tube radius, R, is replaced by a. Furthermore, expressions for dp/dz, μ(r), H_T, and H_D, given by Eqs. 2, 3, and 5, also apply in microvessels if R is replaced by a in Eqs. 2 and 3.
Assuming that red cells cannot penetrate the ESL (12), we model flow in the porous layer with the Brinkman equation (15). Under these flow conditions, the axial fluid velocity component, $v_z^f$, in the porous layer is governed by

$$\frac{\mu_a}{r} \frac{d}{dr} \left( r \frac{dv_z^f}{dr} \right) = \frac{dp}{dz} + K v_z^f, \quad a < r < R,$$

where $\mu_a = \mu(a)$ is the viscosity at the interface between the ESL and blood in the free lumen, and $K$ is the spatially averaged effective hydraulic resistivity of the layer, which is inversely proportional to its permeability.

A generalization of Eq. 4 for the relative apparent viscosity, $\eta_{rel}$, in microvessels is found to be

$$\eta_{rel} = -\frac{1}{8G(\alpha)} \left( \int_0^\alpha \left( 2 - \frac{1}{G(\alpha)} \int_{r/R}^\alpha \frac{\sigma d\sigma}{\mu(\sigma)/\mu_a} \right) \tilde{r} d\tilde{r} + (1 - \alpha^2)\bar{v}^f \right)^{-1}$$

where $\alpha = a/R$ and $\bar{v}^f$ is related to the dimensionless mean velocity of plasma flow through the porous layer (1). The function $G(r)$ and the quantity $\bar{v}^f$ are given in ref. 1 and depend parametrically only upon the dimensionless ESL thickness, $1 - \alpha$, and the dimensionless hydraulic resistivity of the ESL, $KR^2/\mu_a$.

**Procedure for Obtaining the Velocity Distribution from the $\mu$-PIV Data.** We accounted for the hydrodynamic interaction, arising between the microsphere and the vessel/tube wall, that causes the translational speed of the center of a microsphere very near the wall to lag the velocity a fluid particle would have at the same radial distance from the wall if the microsphere were not present in the flow. Following a previously described method (14), the three-dimensional analyses of the free motion of a neutrally buoyant sphere in a uniform shear field adjacent to a planar confining boundary (16) or a Brinkman half space (17) were used to infer, from the measured translational speed of each microsphere, the true fluid particle velocity that would arise in the absence of the particle tracer. The velocity profile over the vessel cross-section was extracted from the estimated fluid particle velocities rather than directly from the measured microsphere velocities.

Because not all of the recorded beads travel in the midsagittal plane, we used a previously described monotonic filter (1,14) and considered only the fastest beads at a given measured radial...
location, where it is understood that a bead in the midsagittal plane travels faster than any other bead at that measured radial location. Only monotonically filtered data were included in the analysis.

Following methods described in ref. 1, an axisymmetric velocity profile, $v_z(r)$, was extracted from each µ-PIV data set. All velocity profiles in vitro and in vivo were obtained using the function defined in ref. 1, which identically satisfies the momentum equation and boundary conditions, and is given by

$$v_{\text{fit}}(r) = v_{\text{max}} \left( 2G(\alpha) - \int_{r/R}^{\alpha} f(\sigma) \, d\sigma \right) \left( 2G(\alpha) - \int_{0}^{\alpha} f(\sigma) \, d\sigma \right)^{-1}, \quad 0 < r/R < \alpha. \quad (9)$$

Here, $f(\sigma) = \sigma(1 - c_1 \sinh c_2 \alpha) + \alpha c_1 \sinh c_2 \sigma$ is the fitting function and $c_1$ and $c_2$ are found by means of nonlinear-regression analysis that uses Eq. 9 to minimize the least-squares error in the fit to the monotonically filtered data points identified above. The parameter $v_{\text{max}}$, corresponding to the axial centerline velocity, is found by solving Eq. 9 for $v_{\text{max}}$, substituting for $r$ and $v_{\text{fit}}$, the measured radial position and velocity of each microsphere (from the monotonically filtered data set after correcting for fluid drag), and then taking the average of those values. It should be noted that the first term on the right side of Eq. 9 represents the slip velocity at $r = \alpha$. For flow in a glass tube, $\alpha = 1$, and because $G(1) = 0 \ (1)$, the slip velocity in Eq. 9 vanishes and Eq. 9 satisfies the no-slip condition at the tube wall. The function $v_{\text{fit}}$ and its derivatives are continuous on $0 \leq r^* \leq \alpha$.

The primary error source in our prediction of $dp/dz$ is attributable to the uncertainty in our estimate of wall shear rate in vitro, because this quantity depends on the first derivative of our fit to the data. We have found that this quantity can be accurately estimated from the data used to extract the velocity distribution as long as (i) the true radial position of at least one particle tracer is within a distance of $\approx 0.1R$ from the tube wall, (ii) at least one particle tracer is within a distance of $\approx 0.2R$ from the tube centerline, and (iii) no two adjacent particle tracers are more than a distance of $\approx 0.4R$ apart if either one of the two tracers has a radial position, $r$, of more than $\approx 0.5R$. Any data set that did not meet all three of these criteria (after the optical correction procedure) was excluded from our analysis. Furthermore, in order to achieve sufficient spatial resolution to accurately predict the velocity distribution near the tube wall, we have found that the microsphere-to-vessel diameter
ratio should be less than $\approx 2\%$.

**Materials and Methods for Glass-Tube Experiments.** Perfusion experiments were performed at room temperature ($23–24^\circ$C), which was continuously monitored at the microscope stage. Capillary tubes were pretreated with 1% Tween 20 in PBS for 2 hours to prevent cell adhesion to the tube wall. The downstream reservoir and silastic tubing were prefilled with degassed saline, and all air bubbles were removed. The fluids were perfused through each glass tube in the following order: saline, plasma, and red-cell suspensions. A high perfusion pressure ($\approx 100 \text{ cm H}_2\text{O}$) was applied for 1 minute at the start of each new perfusate. The perfusion pressure was quickly reduced to establish the target perfusion pressure. The velocity was measured $\approx 1.5 \text{ cm}$ from the inlet of the glass tube, and the flow was measured for 1–2 minutes at each perfusion pressure.

Transillumination was maintained to keep the luminal glass capillary wall clearly visible. The midsagittal plane was defined as corresponding to the focal plane at which the contrast of the edge of the intraluminal wall reversed (14). The diameters of the upstream and downstream ends of the glass tube were determined by end-on microscopy. Capillary tubes were immersed in phthalic acid dibutyl ester (ND = 1.48, Sigma), which has an index of refraction that effectively eliminates optical refraction at the outer tube wall. Optical refraction at the inner tube wall, which arises from the mismatch in the refractive indices of the perfusate and the glass, introduces an optical artifact, as manifested by the disparity between the measured and actual radial positions of the microsphere. This disparity increases with increasing radial position. Applying Snell’s law, the disparity, $\delta x$, between the measured radial position, $d_m$ (open symbols in Figs. 1 and 5–14), and actual radial position, $d_a$ (closed symbols in Figs. 1 and 5–14), of a microsphere in the midsagittal plane of the glass capillary tube is given by (14)

$$
\delta x = d_m - d_a = \sqrt{d_m(2R - d_m)} \tan \left( \sin^{-1} \left( \frac{N_g}{N_p} \sin \theta_1 \right) - \theta_1 \right)
$$

(10)

where $N_g$ and $N_p$ are the indices of refraction of the glass tube wall and perfusate, respectively, and

$$
\theta_1 = \frac{\pi}{2} - \tan^{-1} \left( \frac{\sqrt{d_m(2R - d_m)}}{R - d_m} \right).
$$

(11)
The refractive indices of plasma and saline were determined by using a hand-held refractometer (Atago, Kirkland, WA), and the viscosity of plasma was determined by using a capillary viscometer (Cannon-Manning Semimicro Viscometer, Cannon Instrument, State College, PA).

Table 1. Quantities for 12 venules (Figs. 15–26) in the mouse cremaster muscle

<table>
<thead>
<tr>
<th>Figure</th>
<th>$D$ (µm)</th>
<th>$R - a$ (µm)</th>
<th>$-dp/dz$ (dyn/cm³)</th>
<th>$\eta_{rel}$</th>
<th>$\dot{\gamma}(a)/\dot{\gamma}_P(R)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>34.5</td>
<td>0.59</td>
<td>20,705</td>
<td>5.30</td>
<td>6.4</td>
</tr>
<tr>
<td>16</td>
<td>38.0</td>
<td>0.71</td>
<td>13,692</td>
<td>5.27</td>
<td>6.7</td>
</tr>
<tr>
<td>17</td>
<td>32.8</td>
<td>0.52</td>
<td>17,257</td>
<td>4.01</td>
<td>5.7</td>
</tr>
<tr>
<td>18</td>
<td>24.8</td>
<td>0.63</td>
<td>16,254</td>
<td>4.97</td>
<td>6.9</td>
</tr>
<tr>
<td>19</td>
<td>33.5</td>
<td>0.60</td>
<td>18,798</td>
<td>4.55</td>
<td>6.1</td>
</tr>
<tr>
<td>20</td>
<td>40.7</td>
<td>0.44</td>
<td>7,688</td>
<td>4.82</td>
<td>6.4</td>
</tr>
<tr>
<td>21</td>
<td>24.0</td>
<td>0.37</td>
<td>20,074</td>
<td>2.44</td>
<td>3.4</td>
</tr>
<tr>
<td>22</td>
<td>30.4</td>
<td>0.29</td>
<td>4,448</td>
<td>1.82</td>
<td>2.2</td>
</tr>
<tr>
<td>23</td>
<td>38.9</td>
<td>0.49</td>
<td>5,225</td>
<td>2.63</td>
<td>3.1</td>
</tr>
<tr>
<td>24</td>
<td>32.5</td>
<td>0.46</td>
<td>6,549</td>
<td>3.79</td>
<td>4.7</td>
</tr>
<tr>
<td>25</td>
<td>42.9</td>
<td>0.45</td>
<td>6,549</td>
<td>2.57</td>
<td>2.8</td>
</tr>
<tr>
<td>26</td>
<td>36.8</td>
<td>0.61</td>
<td>5,281</td>
<td>3.65</td>
<td>4.4</td>
</tr>
<tr>
<td>Average</td>
<td>34.2</td>
<td>0.51</td>
<td>11,877</td>
<td>3.82</td>
<td>4.9</td>
</tr>
<tr>
<td>Standard Error</td>
<td>1.7</td>
<td>0.04</td>
<td>1,870</td>
<td>0.35</td>
<td>0.5</td>
</tr>
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</table>

Quantities were calculated based on a hydraulic resistivity of the endothelial surface layer (ESL) of $K = 10^9$ dyn·s/cm⁴.
Table 2. Percentage of decrease in $\mu^*_T$, $\mu^*_D$, and $\eta_{\text{rel}}$ in five cremaster-muscle venules (Figs. 27–31) after systemic hemodilution in three mice

<table>
<thead>
<tr>
<th>Figure</th>
<th>% decrease in $\mu^*_T$</th>
<th>% decrease in $\mu^*_D$</th>
<th>% decrease in $\eta_{\text{rel}}$</th>
<th>% decrease in $H_{\text{sys}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>21.0</td>
<td>22.7</td>
<td>4.7</td>
<td>34.4</td>
</tr>
<tr>
<td>28</td>
<td>48.0</td>
<td>47.2</td>
<td>42.3</td>
<td>34.6</td>
</tr>
<tr>
<td>29</td>
<td>39.4</td>
<td>43.5</td>
<td>26.0</td>
<td>34.6</td>
</tr>
<tr>
<td>30</td>
<td>22.2</td>
<td>27.5</td>
<td>23.5</td>
<td>34.4</td>
</tr>
<tr>
<td>31</td>
<td>36.7</td>
<td>40.6</td>
<td>29.2</td>
<td>29.4</td>
</tr>
<tr>
<td>Average</td>
<td>33.5</td>
<td>36.3</td>
<td>25.1</td>
<td>33.5</td>
</tr>
<tr>
<td>Standard Error</td>
<td>5.2</td>
<td>4.8</td>
<td>6.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Quantities were calculated based on a hydraulic resistivity of the endothelial surface layer (ESL) of $K = 10^9 \text{ dyn} \cdot \text{s/cm}^4$. 
Fig. 5. (A) Optically corrected fluorescent microparticle image velocimetry (µ-PIV) data (filled circles) and raw µ-PIV data (open circles) obtained from a 50.7-µm-diameter glass tube steadily perfused with washed human red cells suspended in plasma. The measured pressure head, Δp, is 22 cm H₂O and the measured discharge hematocrit, H_D, is 55%. Superimposed on the µ-PIV data in A are axisymmetric velocity distributions, v_z(r), extracted from the data. (B) Distributions in shear rate (blue) and shear stress (red) over the tube cross section, corresponding to the velocity distributions shown in A. (C) Predicted distributions in the normalized viscosity, μ(r)/μ_water, derived (1) by using the analytical expression for μ(r) (see Supporting Text). (D) Variation in the normalized least-squares error, E*, associated with the fit to the µ-PIV data shown in A as a function of the thickness, R−a, of a fictitious wall layer that retards plasma flow completely. The minimum value of E* corresponds to a layer of zero thickness. (E) Measured tube diameter, D, and discharge hematocrit, H_D; measured and predicted values of the axial pressure gradient, dp/dz; empirically estimated (3) and predicted values of the relative apparent viscosity, η_reel; and the ratio of the predicted wall shear rate, ̇γ(R), to the wall shear rate, ̇γ_p(R), of a Poiseuille flow having the centerline velocity shown in A. Percentages in parentheses under each of the predicted values listed in the table correspond to the percentage of difference between measured (or empirically estimated) and predicted values.


Fig. 6. Same as Fig. 5, except Δp = 19 cm H₂O.

Fig. 7. Same as Fig. 5, except Δp = 15 cm H₂O.

Fig. 8. Same as Fig. 5, except Δp = 13 cm H₂O, and H_D = 46%.

Fig. 9. Same as Fig. 5, except Δp = 12.5 cm H₂O, H_D = 33.5%, and D = 54.2 µm.

Fig. 10. Same as Fig. 5, except Δp = 10 cm H₂O, H_D = 33.5%, and D = 54.2 µm.
Fig. 11. Same as Fig. 5, except $\Delta p = 12.7$ cm H$_2$O, $H_D = 17.6\%$, and $D = 54.2$ $\mu$m.

Fig. 12. Same as Fig. 5, except $\Delta p = 5$ cm H$_2$O, $H_D = 17.6\%$, and $D = 54.2$ $\mu$m.

Fig. 13. Same as Fig. 5, except $\Delta p = 10$ cm H$_2$O, $D = 54.2$ $\mu$m, and the perfusate is saline.

Fig. 14. Same as Fig. 5, except $\Delta p = 6$ cm H$_2$O, $D = 54.2$ $\mu$m, and the perfusate is saline.

Fig. 15. Intravital fluorescent $\mu$-PIV data and predicted velocity profile (A), shear-rate and shear-stress profiles (B), and viscosity profile (C) in a 34.5-$\mu$m-diameter venule of the mouse cremaster muscle. Curves have the same interpretation as those shown in Fig. 5. The shaded region near the wall of each vessel represents the endothelial surface layer (ESL). Predicted velocity distribution in the ESL assumes a Brinkman medium (1–3) having a hydraulic resistivity, $K = 10^9$ dyn·s/cm$^4$. (D) Variations in the normalized least-squares error, $E^*$, associated with the fit to the $\mu$-PIV data shown in A as a function of the estimated ESL thickness, $R - a$. The three curves show results for $K = 10^9$ dyn·s/cm$^4$ (blue), $K = 10^{10}$ dyn·s/cm$^4$ (red), and the case of no flow through the layer, $K \to \infty$ (black). Estimated layer thickness for each value of $K$ corresponds to the value of $R - a$ associated with the local minimum in each of the corresponding curves. (E) Measured vessel diameter, $D$; estimated ESL thickness, $R - a$, corresponding to $K = 10^9$ dyn·s/cm$^4$; predicted axial pressure gradient, $dp/dz$; predicted relative apparent viscosity, $\eta_{rel}$; and the ratio of the predicted interfacial shear rate, $\dot{\gamma}(a)$, to the wall shear rate, $\dot{\gamma}_P(R)$, of a Poiseuille flow having the centerline velocity shown in A.


Fig. 16. Same as Fig. 15, except $D = 38.0$ $\mu$m.

Fig. 17. Same as Fig. 15, except $D = 32.8$ $\mu$m.
Fig. 18. Same as Fig. 15, except $D = 24.8 \, \mu m$.

Fig. 19. Same as Fig. 15, except $D = 33.5 \, \mu m$.

Fig. 20. Same as Fig. 15, except $D = 40.7 \, \mu m$.

Fig. 21. Same as Fig. 15, except $D = 24.0 \, \mu m$.

Fig. 22. Same as Fig. 15, except $D = 30.4 \, \mu m$.

Fig. 23. Same as Fig. 15, except $D = 38.9 \, \mu m$.

Fig. 24. Same as Fig. 15, except $D = 32.5 \, \mu m$.

Fig. 25. Same as Fig. 15, except $D = 42.9 \, \mu m$.

Fig. 26. Same as Fig. 15, except $D = 36.8 \, \mu m$.

Fig. 27. Intravital fluorescent $\mu$-PIV data with predicted velocity profiles ($A$ and $C$) and normalized viscosity profiles ($B$ and $D$) in a venule (diameter, $\approx 36 \, \mu m$) of the mouse cremaster muscle before ($A$ and $B$) and after ($C$ and $D$) systemic hemodilution. Curves have the same interpretation as those shown in Fig. 1. The shaded region near the vessel wall represents the ESL, where the ESL is modeled as a Brinkman medium (1–3) having a hydraulic resistivity, $K = 10^9 \, \text{dyn-s/cm}^4$. The thickness of the ESL is estimated by minimizing the normalized least-squares error associated with the fit to the $\mu$-PIV data, as described in ref. 3. ($E$) Percentage of decrease after systemic hemodilution in $\mu_T^*$, $\mu_D^*$, $\eta_{rel}$, and the systemic hematocrit, $H_{sys}$. Parameters before ($F$) and after ($G$) hemodilution include the measured vessel diameter, $D$; the estimated ESL thickness, $R - a$, corresponding to $K = 10^9 \, \text{dyn-s/cm}^4$; the predicted axial pressure gradient, $dp/dz$; the predicted relative apparent viscosity, $\eta_{rel}$; and the ratio of the predicted interfacial shear rate, $\dot{\gamma}(a)$, to the wall shear rate, $\dot{\gamma}_P(R)$, of a Poiseuille flow having the centerline velocity associated with the profiles in $A$ and $C$. 


**Fig. 28.** Same as Fig. 27, except $D \approx 47 \mu m$.

**Fig. 29.** Same as Fig. 27, except $D \approx 40 \mu m$.

**Fig. 30.** Same as Fig. 27, except $D \approx 42 \mu m$. Note that the ordinate scales for the velocity profiles differ in A and C.

**Fig. 31.** Same as Fig. 27, except $D \approx 41.5 \mu m$. Note that the ordinate scales for the velocity profiles differ in A and C.
Human red-cell suspension in vitro

Fig. 5.
Human red-cell suspension in vitro

Fig. 6.
Human red-cell suspension in vitro

\[ \tau \text{(dyn/cm}^2\text{)} \]

\[ \mu \text{(r)/\mu water} \]

\[ \gamma \text{(s}^{-1}\text{)} \]

\[ \eta_{rel} \]

\[ \frac{\gamma(R)}{\gamma_p(R)} \]

**Fig. 7.**

<table>
<thead>
<tr>
<th>D = 50.7 \mu m</th>
<th>- \frac{dp}{dz} \text{(dyn/cm}^3\text{)}</th>
<th>\eta_{rel}</th>
<th>\frac{\gamma(R)}{\gamma_p(R)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>H_D = 54.8</td>
<td>5.347</td>
<td>2.53</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>(5.392 \text{ (0.8%)}</td>
<td>3.35</td>
<td>(32%))</td>
</tr>
</tbody>
</table>

meas. | pred. | emp. | pred. |
Human red-cell suspension in vitro

Fig. 8.
Human red-cell suspension in vitro

Fig. 9.

<table>
<thead>
<tr>
<th>D = 54.2 \mu m</th>
<th>\frac{\partial P}{\partial z} (\text{dyn/cm}^3)</th>
<th>\eta_{rel}</th>
<th>\frac{\gamma(R)}{\eta_{P}(R)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>meas.</td>
<td>pred.</td>
<td>emp.</td>
<td>pred.</td>
</tr>
<tr>
<td>4.874</td>
<td>9.306</td>
<td>1.68</td>
<td>2.90</td>
</tr>
</tbody>
</table>

\[ (99\%) \quad (73\%) \]
Human red-cell suspension in vitro

Fig. 10.
Human red-cell suspension in vitro

Fig. 11.
Human red-cell suspension in vitro

Fig. 12.
Saline in vitro

![Graph showing experimental data and theoretical predictions.](image)

<table>
<thead>
<tr>
<th>$D$ (µm)</th>
<th>$-\frac{dp}{dz}$ (dyn/cm³)</th>
<th>$\frac{\gamma(K)}{\gamma_F(K)}$</th>
</tr>
</thead>
<tbody>
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<td>meas.</td>
<td>54.2</td>
<td>3.736</td>
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<tr>
<td>pred.</td>
<td>54.2</td>
<td>3.809 (2%)</td>
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Fig. 13.
Fig. 14:

<table>
<thead>
<tr>
<th>$D$ (µm)</th>
<th>$-\frac{dp}{dz}$ (dyn/cm³)</th>
<th>$\frac{\gamma R}{\gamma_p R}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>meas.</td>
<td>pred.</td>
<td></td>
</tr>
<tr>
<td>54.2</td>
<td>2.236</td>
<td>2.218 (0.8%)</td>
</tr>
</tbody>
</table>
Mouse whole blood in vivo

Figure 15.
Fig. 16.
Fig. 17.
**Mouse whole blood in vivo**

- **A**
  - Plot of $v_z$ (µm/s) vs $r$ (µm)
- **B**
  - Plot of $\dot{\gamma}$ (s$^{-1}$) vs $r$ (µm)
- **C**
  - Plot of $\mu(r)/\mu_{\text{water}}$ vs $r$ (µm)
- **D**
  - Graph of $E^*$ vs estimated ESL thickness, $R-a$ (µm)
  - Four curves for different $K$ values:
    - $K = 10^{10}$ dyn·s·cm$^{-4}$
    - $K = 10^{9}$ dyn·s·cm$^{-4}$
- **E**
  - Table:
    - | $D$ (µm) | $R-a$ (µm) | $-\frac{dp}{dz}$ (dyn/cm$^3$) | $\eta_{rel}$ | $\frac{\gamma(R)}{\gamma_p(R)}$ |
    - | 24.8 | 0.63 | 16,254 | 4.97 | 6.9 |

---

**Fig. 18.**
Mouse whole blood in vivo

Fig. 19.
Estimated ESL thickness, $R-a$ (µm)

Mouse whole blood in vivo

Fig. 20.
**Fig. 21.**
Mouse whole blood in vivo

Fig. 22.
Mouse whole blood in vivo

Fig. 23.
Mouse whole blood in vivo

Fig. 24.
**Mouse whole blood in vivo**

**Fig. 25.**
Mouse whole blood in vivo

Fig. 26.
Mouse whole blood in vivo before hemodilution

Fig. 27.

<table>
<thead>
<tr>
<th>% decrease in ( \mu_T )</th>
<th>% decrease in ( \mu_D )</th>
<th>% decrease in ( \eta_{rel} )</th>
<th>% decrease in ( H_{sys} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{1}{A} \int_A \int_A \frac{\mu(r)}{r} , dA )</td>
<td>( \frac{1}{A} \int_A \int_A \frac{\mu(r)}{r} , \nu_z(r) , dA )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.0</td>
<td>22.7</td>
<td>4.7</td>
<td>34.4</td>
</tr>
</tbody>
</table>

F Parameters before hemodilution

<table>
<thead>
<tr>
<th>( D ) (( \mu m ))</th>
<th>( R - a ) (( \mu m ))</th>
<th>( - \frac{dp}{\pi} ) (dyn/cm(^3))</th>
<th>( \eta_{rel} )</th>
<th>( \frac{\gamma(R)}{\gamma_D / R} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.3</td>
<td>0.53</td>
<td>12,891</td>
<td>2.56</td>
<td>3.1</td>
</tr>
</tbody>
</table>

G Parameters after hemodilution

<table>
<thead>
<tr>
<th>( D ) (( \mu m ))</th>
<th>( R - a ) (( \mu m ))</th>
<th>( - \frac{dp}{\pi} ) (dyn/cm(^3))</th>
<th>( \eta_{rel} )</th>
<th>( \frac{\gamma(R)}{\gamma_D / R} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.0</td>
<td>0.60</td>
<td>8,799</td>
<td>2.44</td>
<td>2.7</td>
</tr>
</tbody>
</table>
Mouse whole blood in vivo before hemodilution

Mouse whole blood in vivo after hemodilution

<table>
<thead>
<tr>
<th>% decrease in $\mu_T$</th>
<th>% decrease in $\mu_D$</th>
<th>% decrease in $\eta_{rel}$</th>
<th>% decrease in $H_{sys}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\left( \frac{1}{2} \int_A \mu(r) dA \right)$</td>
<td>$\left( \frac{1}{2} \int_A \mu_D(r) v_z(r) dA \right)$</td>
<td>48.0</td>
<td>47.2</td>
</tr>
</tbody>
</table>

Parameters before hemodilution

<table>
<thead>
<tr>
<th>$D$ (µm)</th>
<th>$R - a$ (µm)</th>
<th>$-\frac{dp}{dz}$ (dyn/cm$^3$)</th>
<th>$\eta_{rel}$</th>
<th>$\frac{\gamma(R)}{\gamma_p(R_p)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>47.0</td>
<td>0.59</td>
<td>4,492</td>
<td>2.72</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Parameters after hemodilution

<table>
<thead>
<tr>
<th>$D$ (µm)</th>
<th>$R - a$ (µm)</th>
<th>$-\frac{dp}{dz}$ (dyn/cm$^3$)</th>
<th>$\eta_{rel}$</th>
<th>$\frac{\gamma(R)}{\gamma_p(R_p)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>47.3</td>
<td>0.84</td>
<td>2,702</td>
<td>1.57</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Fig. 28.
Mouse whole blood in vivo before hemodilution

Mouse whole blood in vivo after hemodilution

E

<table>
<thead>
<tr>
<th>% decrease in $\mu_T$</th>
<th>% decrease in $\mu_D$</th>
<th>% decrease in $\eta_{rel}$</th>
<th>% decrease in $H_{sys}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\left( \frac{1}{A} \int_{A} \frac{\mu(r)}{\mu_{water}} , dA \right)$</td>
<td>$\left( \frac{1}{Q} \int_{A} \frac{\mu(r) \cdot v_{z}(r)}{\mu_{water}} , dA \right)$</td>
<td>26.0</td>
<td>34.6</td>
</tr>
</tbody>
</table>

F

Parameters before hemodilution

<table>
<thead>
<tr>
<th>$D$ ($\mu$m)</th>
<th>$R$ -- $a$ ($\mu$m)</th>
<th>$-\frac{dp}{dz}$ (dyn/cm$^3$)</th>
<th>$\eta_{rel}$</th>
<th>$\frac{\gamma(R)}{\gamma_{P}(R)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.2</td>
<td>0.82</td>
<td>8,724</td>
<td>3.08</td>
<td>3.4</td>
</tr>
</tbody>
</table>

G

Parameters after hemodilution

<table>
<thead>
<tr>
<th>$D$ ($\mu$m)</th>
<th>$R$ -- $a$ ($\mu$m)</th>
<th>$-\frac{dp}{dz}$ (dyn/cm$^3$)</th>
<th>$\eta_{rel}$</th>
<th>$\frac{\gamma(R)}{\gamma_{P}(R)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.9</td>
<td>0.64</td>
<td>4,386</td>
<td>2.28</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Fig. 29.
Mouse whole blood in vivo before hemodilution

![Graph A](image1.png)

Mouse whole blood in vivo after hemodilution

![Graph B](image2.png)

<table>
<thead>
<tr>
<th>E</th>
<th>% decrease in $\mu_T$</th>
<th>% decrease in $\mu_D$</th>
<th>% decrease in $\eta_{rel}$</th>
<th>% decrease in $H_{sys}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Integration" /></td>
<td>22.2</td>
<td>27.5</td>
<td>23.5</td>
<td>34.4</td>
</tr>
</tbody>
</table>

Parameters before hemodilution

<table>
<thead>
<tr>
<th>F</th>
<th>$D$ ($\mu$m)</th>
<th>$R - a$ ($\mu$m)</th>
<th>$-\frac{dp}{dx}$ (dyn/cm$^3$)</th>
<th>$\eta_{rel}$</th>
<th>$\frac{\gamma(R)}{\gamma_p(R)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.4</td>
<td>0.72</td>
<td>26,014</td>
<td>4.73</td>
<td>5.3</td>
<td></td>
</tr>
</tbody>
</table>

Parameters after hemodilution

<table>
<thead>
<tr>
<th>G</th>
<th>$D$ ($\mu$m)</th>
<th>$R - a$ ($\mu$m)</th>
<th>$-\frac{dp}{dx}$ (dyn/cm$^3$)</th>
<th>$\eta_{rel}$</th>
<th>$\frac{\gamma(R)}{\gamma_p(R)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>41.6</td>
<td>0.02</td>
<td>4,524</td>
<td>3.62</td>
<td>4.2</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 30.
Mouse whole blood in vivo before hemodilution

\[ \frac{1}{A} \int_{A} \mu_{r} \frac{\partial r}{\partial A} \, dA \]

% decrease in \( \mu_{T} \)

\[ \frac{1}{A} \int_{A} \mu_{r} \frac{\partial r}{\partial A} \, dA \]

% decrease in \( \mu D \)

\[ \frac{1}{A} \int_{A} \mu_{r} \frac{\partial r}{\partial A} \, dA \]

% decrease in \( \eta_{rel} \)

% decrease in \( H_{sys} \)

\[ \frac{1}{A} \int_{A} \mu_{r} \frac{\partial r}{\partial A} \, dA \]

Parameters before hemodilution

<table>
<thead>
<tr>
<th>( D (\mu m) )</th>
<th>( R - a (\mu m) )</th>
<th>( -\frac{dp}{dz} ) (dyn/cm(^3))</th>
<th>( \eta_{rel} )</th>
<th>( \frac{\gamma (R)}{\gamma_{P}(R)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.8</td>
<td>0.78</td>
<td>20,462</td>
<td>5.04</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Parameters after hemodilution

<table>
<thead>
<tr>
<th>( D (\mu m) )</th>
<th>( R - a (\mu m) )</th>
<th>( -\frac{dp}{dz} ) (dyn/cm(^3))</th>
<th>( \eta_{rel} )</th>
<th>( \frac{\gamma (R)}{\gamma_{P}(R)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.4</td>
<td>0.25</td>
<td>5,507</td>
<td>3.57</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Fig. 31.