Regulation of capillary hydraulic conductivity in response to an acute change in shear

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Regulation of capillary hydraulic conductivity in response to an acute change in shear. Am J Physiol Heart Circ Physiol 289: H2126–H2135, 2005. First published July 1, 2005; doi:10.1152/ajpheart.01270.2004.—The effects of mechanical perturbations (shear stress, pressure) on microvascular permeability have been examined in micropipette-cannulated vessels or in endothelial monolayers in vitro. The objective of this study was to determine whether acute changes in blood flow shear stress might influence measurements of hydraulic conductivity (Lp) in autoperfused microvessels in vivo. Rat mesenteric microvessels were observed via intravital microscopy. Occlusion of a third-order arteriole with a micropipette was used to divert and increase flow through a nonoccluded capillary or fourth-order arteriolar branch. Transvascular fluid filtration rate in the branching vessel was measured with a Landis technique. Flow (shear)-induced increases in Lp disappeared within 20–30 s of the removal of the shear and could be eliminated with nitric oxide synthase inhibition. The shear-induced increase in Lp was greater in capillaries compared with terminal arterioles. An acute change in shear may regulate Lp by a nitric oxide-dependent mechanism that displays heterogeneity within a microvascular network.

NEARLY EIGHTY YEARS AGO, in 1927, Landis (25) published a novel technique whereby movement of red blood cells in a micropipette-occluded capillary could be used to calculate transvascular filtration rate. His technique, and the modifications by Michel et al. (31) in 1974, remain the most widely used method to investigate regulation of fluid filtration of individual microvessels in vivo.

According to Starling’s hypothesis, transvascular fluid flux across the endothelial transport barrier is regulated by a change in either pressure gradient (hydrostatic or oncotic) across the vascular wall or permeability [oncotic reflection coefficient (σ) or hydraulic conductivity (Lp)]. Application of chemical agents increases (e.g., histamine, thrombin; reviewed in Ref. 30) or decreases (e.g., cyclic AMP; Ref. 1) Lp and fluid filtration rate. Mechanical factors also may affect Lp because endothelial cells can sense and trigger mechanotransduction in response to changes in blood flow shear stress and hydrostatic pressure (4, 5).

Using the micropipette occlusion technique in autoperfused rather than cannulated capillaries, Landis described how the fluid filtration rate was most rapid in the initial seconds following occlusion, becoming significantly slower in less than 1 min. This was attributed at least in part to the possibility that plasma oncotic pressure could increase with time in an occluded vessel as a result of greater efflux of water than of protein. However, another consideration is that the vessel occlusion includes an abrupt change in flow-induced shear stress and pressure, both of which could have a time-dependent effect on Lp.

As a microvessel is occluded, blood flow decreases from its basal value to essentially zero. Several studies have shown that transvascular exchange can be affected by a change in perfusion rate (14, 17, 19, 32, 33, 43). A small-pore model has been proposed as a possible mechanism to account for flow-dependent transport of small solutes in cannulated vessels (32); moreover, there have been several recent studies of shear-induced increases in fluid filtration performed in vitro (3, 6, 37) and in vivo (27, 40, 41). The mechanism by which shear forces could increase Lp appear to be dependent on nitric oxide (NO) (3, 15, 24). Therefore, when a capillary stops flowing, it might be expected that Lp could decline in a matter of seconds as shear-induced production of NO is attenuated.

Another mechanical factor associated with microvessel occlusion is the step increase in hydrostatic pressure. For example, when a capillary is occluded at its downstream end, pressure throughout the vessel increases to that of the feeding arteriole. Recent studies (7, 38) indicate that a step increase in hydrostatic pressure causes a decrease in Lp in what has been called a “sealing effect.” However, the time courses of the changes in Lp in these studies were over a period of minutes to hours.

The effects of shear on Lp have been examined previously in micropipette-cannulated vessels or in endothelial monolayers in vitro. The primary objective of the current study was to determine whether acute changes in blood flow shear might influence measurements of Lp in autoperfused, rather than cannulated, microvessels. In addition, a role for NO in shear-mediated changes in Lp was investigated.

MATERIALS AND METHODS

Animal preparation. Animal procedures were approved by an Institutional Animal Care And Use Committee. Male Wistar rats ~2–3 mo old were initially anesthetized with Halothane followed by an intraperitoneal injection of 135 mg/kg thiobutabarbitral (Inactin, catalog no. T-133; Sigma, St. Louis, MO). The right carotid artery was cannulated for a blood pressure monitor (BP-1; World Precision Instruments, Sarasota, FL) and for systemic injection of the euthanasia solution. A segment of the small intestine was exteriorized through a
midline abdominal incision, and the rat was placed on its right side on a Plexiglas board so that a selected section of mesentery could be draped over a glass coverslip glued on a hole centered in the board. The exposed intestine, except for the selected mesenteric section under study, was covered with gauze soaked in bicarbonate-buffered saline (BBS) consisting of 131 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 20 mM NaHCO3, and 3.5 mM CaCl2. After the board was mounted onto the stage of an inverted microscope, the mesentery and intestine were kept moist with a 2 ml/min superfusion of BBS bubbled with a 95% N2-5% CO2 gas mixture and warmed to 37°C. Rectal temperature was maintained near 37°C by positioning an infrared heat lamp over the rat.

Video microscopy. The mesentery was observed through an ×40 objective (Nikon Plan Apo, 0.95 NA) using a 100-W halogen light source, and bright-field images were captured with a color camera (ImageStar IS209; Optical Apparatus, Ardmore, PA). The image was directed into a videocassette recorder, and the taped image was used for playback analysis with an image grabber and image processor (Optimas; Media Cybernetics, Silver Spring, MD) for length and diameter measurements. Microvessel red blood cell velocity (Vv/sec) was measured with live images using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M, College Station, TX).

Measurement of transvascular filtration. Capillary and arteriolar filtration rates (Jv) were measured using a modification of the original Landis technique (25) during micropipette occlusion. The occluder was positioned over a selected fourth-order (A4) arteriole (15–20 μm in diameter) or capillary (6–8 μm in diameter) and carefully lowered onto the vessel by micromanipulator to compress the lumen. The filtration rate was calculated from the decreasing volume (V) between the micropipette and red blood cells that were ~250–400 μm up-stream of the occluder (250–300 μm for capillaries and 300–400 μm for arterioles). During occlusion, red blood cells within the vessel gradually move closer together and toward the occlusion site as the intravascular fluid separating the cells filters across the endothelial barrier into the surrounding tissue. There is also a concomitant change in vessel diameter associated with the myogenic response for an arteriole (21). We assumed uniform circular tube geometry of diameter D and length x, where x is the distance of a marker cell from the occlusion site. The calculation of Jv can then be obtained from a volume balance, where the rate of change in V between a marker cell and the occluder is equal to the rate at which plasma volume is filtered out

\[ J_v = -\frac{dV}{dt} \] (1)

However, because both the position of the marker cell and the diameter change, we employed the chain rule

\[ dV/dt = (\pi D^2 x)/4 \times (dV/dx) \times (dD/dt) \] (2)

With V = πD^2 x/4, the partial derivatives can be computed as dV/dx = πD^2 x/2 and dV/dx = πD^2 x/4. Substituting for these partial derivatives, and then dividing dV/dt by surface area S (S = πDx), we obtained

\[ J_v/S = -(dV/dt)/S = -(1/2) \times (dD/dt) \times (Ddx)/dx \] (3)

The second term on the right-hand side of this equation is the form used in experimental protocols (26) in which vascular diameter is at steady state (that is, when dD/dt = 0).

Measurement of shear rate. Arteriolar and capillary mean fluid velocity were calculated as Vmean = centerline Vmean/correction factor. As suggested by the data of Lipowsky and Zweifach (28), we used a correction factor of 1.6 for arterioles and 1.3 for capillaries. Assuming cylindrical geometry, wall shear rate (SR) can be estimated using the Newtonian definition SR = 8Vmean/D, although because of the particulate nature of blood and the potential influence of the endothelial glyocalyx, the precise value of shear is difficult to determine. In some of our experiments, we induced an increase in shear rate. The normalized increase in shear rate in the same vessel can be expressed as

\[ SR_{shear}/SR_{baseline} = (V_{shear}/V_{baseline}) \times (D_{baseline}/D_{shear}) \] (4)

Starling forces regulating fluid filtration. The effects of hydrostatic and osmotic pressures on fluid filtration are described by Starling’s law

\[ J_v/S = Lp[(P_v - P_a) - \sigma(\pi_v - \pi_a)] \] (5)

where P is the hydrostatic pressure, π is the osmotic pressure, σ is the osmotic reflection coefficient, and the pressure subscripts P and π denote plasma and tissue, respectively. Because the mesentery was exteriorized and superfused with protein-free buffer, tissue hydrostatic (as measured in Ref. 18) and osmotic pressures were negligible. Even with physiological levels of protein in the tissue, filtration values should be unaffected as demonstrated by Hu et al. (16). The equation reduces to

\[ J_v/S = Lp(p_v - \sigma p_a) \] (6)

Method of increasing shear rate and hydraulic conductivity measurements in capillaries. An increase in blood flow velocity through a capillary was induced by occluding a feeding third-order (A3) arteriole downstream of the capillary branch point. As shown in Fig. 1, the occlusion of an A3 arteriole can result in an increase in capillary shear rate (Fig. 1C). However, occlusion of the upstream arteriole also induces an increase in pressure upstream of the occluder and can lead to an increase in capillary pressure. In our previous measurements of A3 arteriolar pressure (21) in the Wistar rat mesentery (same model as the current study), the mean value was 43 mmHg and the pressure increase due to occlusion was 7.4 mmHg. Therefore, it was expected that the pressure increase in the capillary due to arteriolar occlusion would be ~7.4 mmHg with increasing shear. However, the precise value was not measured in the current study and could vary depending on individual resistance and downstream pressure. During the measurement of Jv/S using the modified Landis technique, capillary pressure would be equilibrated to arteriolar pressure (P_a) due to capillary occlusion. In all the Jv/S measurements from capillaries, red blood cell movement due to filtration was measured within 50–100 μm from the arteriolar branch. Baseline Jv/S can be expressed as

\[ (J_v/S)_{baseline} = (L_p/S)_{baseline}(P_a - \sigma p_a) \] (7)

where πa is the osmotic pressure in the capillary. At the higher shear rate, capillary pressure will be increased to P_a + magnitude of pressure increase (ΔP_a) during the measurement of Jv/S, and thus Jv/S at the high shear state can be expressed as

\[ (J_v/S)_{shear} = (L_p/S)_{shear}(P_a + \Delta P_a - \sigma p_a) \] (8)

where σbaseline and σshear refer to osmotic reflection coefficients at baseline and increased shear, respectively.

Dividing Eq. 8 by Eq. 7 and rearranging yields

\[ \frac{(L_p/S)_{shear}}{(L_p/S)_{baseline}} = \frac{(P_a - \sigma p_a)_{baseline}}{(P_a + \Delta P_a - \sigma p_a)} \times \frac{(J_v/S)_{shear}}{(J_v/S)_{baseline}} \] (9)

where P_a = 43 mmHg, ΔP_a = 7.4 mmHg (average value, from Ref. 21), σbaseline = 0.9, and π_a = 15 mmHg (from Ref. 10). The baseline L_p for the capillaries in this study was estimated to be 1.25 × 10^{-7} cm^3 s^{-1} cmH_2O^{-1} using Eq. 7, which is comparable to that reported in postcapillary venules by Rumbaut et al. (mean L_p = 2.3 × 10^{-7} cm^3 s^{-1} cmH_2O^{-1}) using a similar model in the rat mesentery (36). The slightly higher values for venules are consistent with the known increase in microvascular permeability in the arteriole-to-venule direction. σshear was assumed to be 0.9 in the estimation of normalized L_p based on the assumption that the increase in shear rate would not alter σ. However, we do not rule out the possibility that there will be
a change in $\sigma$ with the change in shear rate, and this is addressed in the data analysis. The increase in shear rate in $A_4$ arterioles was induced in the same manner as in capillaries.

**Measurement of Texas red-albumin fluorescence during capillary occlusion.** With fluorescence microscopy, the change in vascular Texas red-labeled bovine serum albumin (TR-albumin) fluorescence intensity was measured to determine changes in capillary protein concentration during capillary occlusion. Before the injection of 15 mg/kg TR-albumin, $J_v/S$ was measured using the Landis technique, and then TR-albumin was injected systemically through the carotid artery. After 2 min, the fluorescence intensity in the middle of the vessel was recorded every 5 s (exposure time < 2 s to limit photobleaching of the dye) for a period of 50 s by using an intensifier gain of 3.5 (Hamamatsu C2400 – 80). An in vitro calibration demonstrated linearity between TR-albumin and fluorescence intensity over the range of concentrations used.

**Experimental protocols.** After duplicate measures were obtained of $J_v/S$ for 50 s each with the use of a modified Landis technique, the micropipette occluder was released to resume blood flow in the capillary. After 10 min, the capillary was exposed to increased flow velocity for 60 s by occluding the feeding $A_3$ arteriole, and then $J_v/S$ was measured twice again from the same capillary. Figure 2 demonstrates the measurement protocol using $J_v/S$ data from five experiments. Measurements of $J_v/S$ for both baseline and high shear were repeated and averaged from the duplicate measures. Whenever an occlusion caused overt damage that precluded subsequent measures of $J_v/S$, the experiment was terminated and the data were not included. The same experimental protocol was used for arteriolar $J_v/S$ measurements, in which $J_v/S$ was measured from $A_4$ arterioles and the feeding $A_3$ arteriole was occluded to induce an acute increase in shear rate through the $A_4$ arteriole. To further investigate whether the shear-induced change in $J_v/S$ was associated with NO, the nitric oxide synthase (NOS) inhibitor nitro-L-arginine methyl ester (L-NAME, 50 $\mu$M; Sigma) was superfused after the baseline $J_v/S$ measurements. After 10-min superfusion of L-NAME, the capillary was exposed to a higher shear rate for 60 s and $J_v/S$ was measured a second time.

**Statistics.** Paired or unpaired $t$-tests were used when two sets of data were compared using Minitab software (State College, PA). Data among groups were compared with the Bonferroni test. Error bars are represent $\pm$SE. Statistical significance was set at $P < 0.05$. 

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**Fig. 1. Method of increasing capillary shear rate.** After the baseline fluid filtration rate ($J_v/S$) measurement, capillary shear rate (SR) was increased for 1 min by occluding the feeding arteriole just downstream of the capillary branch. A: baseline capillary shear rate ($SR_{baseline}$) and pressure ($P_c$) before capillary occlusion. $P$, hydrostatic pressure; $P_a$, arteriolar pressure. B: baseline capillary occlusion for $J_v/S$ measurements; during occlusion, pressure increases to that in the feeding arteriole ($P_a$). C: increased shear rate ($SR_{shear}$) through capillary by arteriolar occlusion that also increases arteriolar pressure ($\Delta P_a$). D: $J_v/S$ measurement at increased shear rate and pressure ($P_a + \Delta P_a$). Open and filled arrows indicate blood flow through arteriole and capillary, respectively.

**Fig. 2. Time course of capillary $J_v/S$ and shear rate ($n = 5$, $SR_{shear}/SR_{baseline} = 1.93 \pm 0.15$, $SR_{shear} = 2.167 \pm 381$ s$^{-1}$, and $SR_{baseline} = 1.169 \pm 237$ s$^{-1}$). Repeat measures of $J_v/S$ were obtained for both baseline and increased shear. The x-axis (time) is not to scale.**
RESULTS

Figure 3 shows the time dependence of \( J_v/S \) after an occlusion (A), the change in \( J_v/S \) (B), and normalized \( L_p \) (C) in response to an acute increase in shear rate \((n = 15; SR_{baseline} = 2.03 \pm 0.08, SR_{shear} = 2.627 \pm 284 \text{ s}^{-1}, \) and SR_{baseline} = 1.327 \pm 147 \text{ s}^{-1})\) in capillaries of rat mesentery. In the baseline measurement of \( J_v/S \), there was a transient decline in which \( J_v/S \) at 50 s of occlusion was only 55 \pm 8\% of the value obtained at 5 s (Fig. 3A). The increase in shear rate was accompanied by an increase in pressure of 17\% (21). With an increase in shear (102\%) and pressure (17\%), \( J_v/S \) increased by 91\% in the initial 5 s of measurement compared with baseline, but only by 25\% by 50 s (Fig. 3B). Assuming a 17\% increase in hydrostatic pressure (from 43.0 to 50.4 mmHg, Ref. 21), Starling’s equation estimates a 24\% increase in \( J_v/S \) with no change in \( L_p \) or \( \sigma \) (with the latter assumed to be 0.9). The time course of \( L_p \) (normalized to baseline; Eq. 9) shows an initial increase followed by transient return to basal values in response to the increase in shear rate and pressure (Fig. 3C). During the initial 10 s, \( L_p \) increased by 53 \pm 4\% from the baseline value \((P < 0.05)\) and then returned to the baseline \( L_p \) after 20–30 s.

A positive correlation was observed between the normalized \( L_p \) and the percentage change in shear rate in the initial 10 s of measurement \((P < 0.001, r^2 = 71.2\% )\), but there was a lack of correlation from time \( \geq 20 \text{ s} \) \((P > 0.05)\) as shown in Fig. 4. Our estimates of normalized \( L_p \) were obtained using the assumption of a consistent 7.4-mmHg increase in arteriolar pressure after occlusion (21). However, we cannot rule out the possibility that the higher changes in shear rate might be accompanied by a larger increase in pressure, thereby resulting in a larger increase in \( J_v/S \). To address this uncertainty, we tested the dependency of the relationship between increase in shear rate \((\% )\) and normalized \( L_p \) \((t = 5 \text{ s})\) on the uncertainty in \( \Delta P_a \) (Table 1). Considering the standard deviation of the pressure change, 2.0 mmHg (21), the magnitude of step increases in pressure induced by arteriolar occlusion was varied from 5.4 to 9.4 mmHg (average value is 7.4 mmHg, see Ref. 21) based on shear rate ranges. When different values of \( \Delta P_a \) were used depending on the percent change in shear rate (Table 1, case B), the slope of the regression plot between percent change in shear rate and normalized \( L_p \) diminished compared with case A (consistent 7.4 mmHg change) but still positively correlated \((P < 0.01)\).

To investigate the effect of NOS inhibition on the shear-induced change in capillary \( J_v/S \), we administered \( \text{L-NAME} \) before and during the increase in shear rate (Fig. 5). Superfusion of 50 \( \mu \text{M} \text{L-NAME} \) significantly attenuated \( J_v/S \) at high shear rate at 5 s, but there was no significant difference at 15, 30, and 50 s (Fig. 5A). Correspondingly, the superfusion of \( \text{L-NAME} \) eliminated the shear-induced increase in \( L_p \) during the initial 10 s following occlusion. In addition, normalized \( L_p \) during baseline conditions was attenuated by 21\% \((P < 0.05)\) after the superfusion of \( \text{L-NAME} \) during the initial 5 s (Fig. 5B).

Similar experiments were performed in small arterioles to investigate the potential influence of the change in shear rate on arteriolar \( J_v/S \) (Fig. 6A) and \( L_p \) (Fig. 6B). The acute increase in shear rate in fourth-order arterioles was almost identical to that of true capillaries (106 \pm 6\% increase in arterioles vs. 102 \pm 8\% increase in capillaries). As shown in Fig. 6B, the time course of \( L_p \) in small arterioles also showed an initial increase from its baseline value (statistically different from baseline, \( P < 0.05 \)) for 10–15 s, followed by a return to baseline by 20 s after the occlusion.

Figure 7 demonstrates the comparison of percent change in \( L_p \) in response to increase in shear rate at 5, 15, 30, and 50 s between arterioles and true capillaries. The increase in \( L_p \) during the initial 5 s in small arterioles was only 23 \pm 4\%, compared with 53 \pm 4\% increases in capillaries.
Fig. 4. Response of \( L_p \) to increased shear for true capillaries. \( A \): normalized \( L_p \) as a function of %change in shear rate (\( n = 14 \)). The line represents the linear regression between normalized \( L_p \) and %change in shear rate (where \( L_p \text{baseline}/L_p \text{ shear} = 0.00364\Delta SR/\% + 1.11 \)). \( B \): slope of the linear regression relation between normalized \( L_p \) and %change in shear rate at different times after capillary occlusion.

As shown in Fig. 3, \( A \) and \( B \), capillary \( J_s/S \) declined with time after an occlusion. However, the rate of decline in the first 15 s of occlusion was four times faster (\( P < 0.05 \)) with elevated shear compared with baseline (Fig. 8A). In contrast, in the presence of L-NAME, the rate of decline of \( J_s/S \) with elevated shear was not statistically different from baseline in the same initial period. Compared with that in capillaries, the rate of \( J_s/S \) decline in arterioles in the first 15 s of occlusion was only twice as great in the high shear case compared with baseline (Fig. 8B).

To investigate the possible contribution of increased protein concentration in the transient response of \( J_s/S \), we measured the fluorescence intensity of TR-albumin during capillary occlusion (Fig. 9). During the initial 50 s, \( J_s/S \) decreased by 45 ± 3% and the intravascular TR-albumin intensity increased by 12 ± 1%. Assuming a capillary hematocrit of 25%, plasma protein concentration can be estimated to increase by 12/(1 – 0.25) = 16%. A 16% increase in protein concentration might account for an ~11% decrease in \( J_s/S \) (details of this estimation are given in DISCUSSION). Light exposure was limited by short 2-s exposure times. However, to investigate whether photobleaching of the dye could underestimate the fluorescence change, we measured the time course of \( J_s/S \) and fluorescence in separate experiments downstream of an occlusion, where filtration rates are minimal (Fig. 9A). If photobleaching occurred, fluorescence intensity downstream of the occlusion would be expected to decrease with time; however, no tendency toward a decrease was observed (Fig. 9B).

In all calculations of normalized \( L_p \), we assumed a constant value of reflection coefficient \((\sigma)\) and values of pressure from a previous study (21) of the same rat strain, age, tissue, and vessel size. To assess the potential uncertainty on the estimation of normalized \( L_p \), we repeated the calculations with various values of pressure and \( \sigma \). Figure 10 demonstrates the sensitivity of the calculation with changing baseline value of pressure in the range of 35–51 mmHg, the magnitude of pressure change in the range of 5.4–9.4 mmHg, and allowing \( \sigma \) to change in the range of 0.6–0.9 after the increase in shear. Assuming an extreme change of pressure and \( \sigma \) (\( P_a = 35 \text{ mmHg}, \Delta P_a = 9.4 \text{ mmHg}, \sigma = 0.6 \)) after the increase in shear, normalized \( L_p \) was estimated to decrease from 1.53 ± 0.04 (when \( P_a = 43 \text{ mmHg}, \Delta P_a = 7.4 \text{ mmHg}, \sigma = 0.9 \) to 1.11 ± 0.03, but still significantly >1 (\( P < 0.05 \)). At the other extreme (when \( P_a = 51 \text{ mmHg}, \Delta P_a = 5.4 \text{ mmHg}, \sigma = 0.9 \)), normalized \( L_p \) in response to shear was estimated to be 1.72 ± 0.05.

**DISCUSSION**

The major finding of the present study is that changes in normalized \( L_p \) were positively correlated with acute changes in shear rate in autoperfused microvessels in rat mesenteric tissue and that the effect disappears within seconds of the elimination of shear. With the modified Landis technique, micropipette occlusion of microvessels reduced shear to essentially zero and allowed a measure of fluid filtration rate \((J_s/S)\). We observed a transient decline in which capillary \( J_s/S \) at 50 s of occlusion was only 55 ± 8% of the value obtained at 5 s (Fig. 3A). When flow was restored for only 60 s before a second occlusion, \( J_s/S \) was fully restored (Fig. 2).

**Table 1. Dependency of relationship between increase in shear rate and normalized hydraulic conductivity on the uncertainty in magnitude of the pressure increase**

<table>
<thead>
<tr>
<th>Increase in Shear Rate</th>
<th>Case A</th>
<th>Case B</th>
</tr>
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<tbody>
<tr>
<td>( \Delta SR &lt; 80% )</td>
<td>( P_a = 43 \text{ mmHg} )</td>
<td>( P_a = 43 \text{ mmHg} )</td>
</tr>
<tr>
<td>( 80% &lt; \Delta SR &lt; 120% )</td>
<td>( \Delta P_a = 7.4 \text{ mmHg} )</td>
<td>( \Delta P_a = 5.4 \text{ mmHg} )</td>
</tr>
<tr>
<td>( \Delta SR &gt; 120% )</td>
<td>( P_a = 43 \text{ mmHg} )</td>
<td>( P_a = 43 \text{ mmHg} )</td>
</tr>
<tr>
<td></td>
<td>( \Delta P_a = 7.4 \text{ mmHg} )</td>
<td>( \Delta P_a = 7.4 \text{ mmHg} )</td>
</tr>
<tr>
<td></td>
<td>( \Delta P_a = 7.4 \text{ mmHg} )</td>
<td>( \Delta P_a = 9.4 \text{ mmHg} )</td>
</tr>
<tr>
<td>Regression</td>
<td>( L_{\text{pixels}}/L_{\text{phase}} = 0.00364(\Delta SR) + 1.11 )</td>
<td>( L_{\text{pixels}}/L_{\text{phase}} = 0.00172(\Delta SR) + 1.32 )</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt;0.001</td>
<td>0.009</td>
</tr>
<tr>
<td>( R^2, % )</td>
<td>71.2</td>
<td>40.4</td>
</tr>
</tbody>
</table>

*Case A* is the same as the results shown in Fig. 4, in which the same values of arteriolar pressure \((P_a)\) and pressure increase \((\Delta P_a)\) were used in the calculation of normalized hydraulic conductivity \((L_p)\) irrespective of changes in the shear rate \((\Delta SR)\). In case *B*, the same values of \( P_a \) were used irrespective of \( \Delta SR \), but different values of \( \Delta P_a \) were used depending on \( \Delta SR \).
The transient nature of $J_v/S$ after occlusion was described in the original study by Landis (25), who used autoperfused vessels as similarly performed in our current study. In contrast, in most studies of buffer-cannulated (rather than autoperfused) microvessels, $L_p$ remains essentially constant throughout the reported period of observation, through at least 30 s of occlusion as described in the study by Michel et al. (31). However, in an exception, Williams (40) specifically described inclusion of data from the first occlusion following vessel cannulation and found a time-dependent decline in $L_p$. In comparison, they found that a relatively stable value of $J_v/S$ was sustained in upstream arteriolar capillaries over 300–400 s. In the same report, the fastest decline in $L_p$ after occlusion was found in venular capillaries: the time-dependent decline in $L_p$ was progressively more substantial going from arterioles to venules, similar to our findings of a greater decline in capillaries vs. terminal arterioles.

There are several possible explanations for the phenomenon of a time-dependent decline in $L_p$ after occlusion. First, and we believe most likely, is that the decline is related to the step reduction in shear (to essentially zero) after occlusion, i.e., $L_p$ could be dependent on shear, and therefore $L_p$ decreases during the occlusion on a time scale similar to the increase in $L_p$ that we find with an increase in shear. However, alternative possibilities that can be discussed are 1) a pressure sealing effect, 2) concentration of vascular protein, 3) buildup of an active agent that decreases $L_p$ during occlusion, and 4) a cell-settling effect.

**Pressure sealing.** During occlusion, intravascular hydrostatic pressure increases almost instantaneously to the pressure...
of the feed vessel. Therefore, a question is whether elevated pressure could cause a “sealing effect” that has been described for endothelial monolayers in vitro (7, 38, 39). Turner (39) observed a decrease in the rate of liquid flow per unit area with time under exposure of constant pressure in monolayers of arterial endothelium. Mechanical and biological mechanisms are known to be involved in the pressure sealing effect. DeMaio et al. (7) suggested that the sealing effect reduces the size of the pathway for water transport by mechanical compression upon pressure application during the initial period of sealing in bovine aortic endothelial cell monolayers. They also observed a significant junctional increase in the tight junction protein zonula occludens-1 after longer periods of pressure application, suggesting the role of a biological mechanism in this phenomenon by which the length or frequency of breaks in the tight junction that constitute the major pathway for water are reduced. However, the contribution of this effect seems negligible, because the pressure sealing effect may occur over a much longer time course of ~30 min compared with our observations over a period of ~30 s.

**Vascular protein.** A second alternative explaining the transient nature of \( J_i/S \) after occlusion is that when the relatively protein-free filtrate leaves the vessel lumen, the vascular protein concentration and associated osmotic pressure increases, thereby decreasing the effective Starling gradient. However, the contribution of this effect is much less than the observed decrease in filtration. For example, in our experiments, the vascular protein increased by ~16% in the initial 50 s after an occlusion. If protein concentration increased from the usual value of ~4.7 g/dl (in this age of rat, Ref. 2, 10) to a value of 5.5 (an increase of 16%), plasma oncotic pressure would be expected to increase from 15 to 18.7 mmHg, according to the equation derived for rat plasma proteins (8). Given that the hydrostatic pressure of the capillary is equal to arterial pressure (43 mmHg) during the occlusion (21), the Starling gradient would decrease only by 11% as a result of the increase in protein concentration (using Eq. 6): \( \left[ \frac{[43 - (0.9 \times 18.7)]}{[43 - (0.9 \times 15)]} \right] = 0.89 \). Therefore, an increase in protein concentration might account for approximately one-fourth of the 45% decrease in \( J_i/S \) that we observed in the first 50 s after an occlusion.

**Mediator accumulation.** A third alternative explaining the decrease in \( J_i/S \) is buildup of an active agent that decreases \( L_p \). During the vascular occlusion, local accumulation of the agent could lead to the decrease in \( L_p \). In addition, more agent would build up during baseline shear than during the faster washout of high shear. However, if this is the case, our L-NAME data suggest that the mediator acts through a NO-mediated pathway (Fig. 5B).
Cell settling. Our tissue preparation involves a horizontal placement of the mesentery for microscopic observation. When a microvessel is occluded, cells within the plasma might settle toward the lower side of the vessel because of gravity. Therefore, a fourth alternative explaining the time-dependent decrease in $J_v/S$ is that cell movement in the axial direction (horizontal; due to filtration) could decline if the cell moves out of the centerline and toward the bottom of the vessel (vertically). If so, measurements of $L_p$ would be expected to decline with time after occlusion. However, the dramatic influence of cell settling would be expected to be the same in both vessel types.

In our experiments, baseline $J_v/S$ was measured, and then, before a second capillary occlusion, we increased baseline capillary shear rate by $\sim 100\%$ by occluding the feeding arteriole just downstream of the capillary branch. This technique also increases arteriolar pressure by 17% (21). With this increase in shear and pressure, $J_v/S$ increased by almost 100% (similar to the increase in shear rate) in the initial 5 s of measurement compared with baseline but only by $\sim 25\%$ by 50 s (Fig. 3B). With shear rates equal to zero during capillary occlusion, the 25% difference at 50 s appears to correspond to the sustained pressure difference due to downstream arteriolar occlusion. We hypothesize that the larger change in shear may be a more important factor than the smaller change in pressure in influencing the initial decline in $J_v/S$ after occlusion.

In this study, a selectively located arteriolar occlusion was used to elicit an increase in blood flow velocity in a nonocluded capillary branch in vivo. After baseline $J_v/S$ was measured, the capillary was exposed to increased flow velocity for 60 s, and then $J_v/S$ was measured again. The limitation of this technique in finding the effect of shear on $L_p$ is that the capillary is in a state of zero shear rate when making measurements with the Landis technique, thereby complicating the interpretations. However, we hypothesized that the increased shear effect would appear at least during the initial seconds of measurement. A similar phenomenon was observed in experiments of shear-dependent vasodilation by using the same technique of arteriolar occlusion (22, 23), where an increase in shear, pressure, $J_v/S$ increased by almost 100% (similar to the increase in shear rate) in the initial 5 s of measurement compared with baseline but only by $\sim 25\%$ by 50 s (Fig. 3B). With shear rates equal to zero during capillary occlusion, the 25% difference at 50 s appears to correspond to the sustained pressure difference due to downstream arteriolar occlusion. We hypothesize that the larger change in shear may be a more important factor than the smaller change in pressure in influencing the initial decline in $J_v/S$ after occlusion.
stress on \( L_p \) in cannulated capillaries of frog mesentery, in which baseline \( L_p \) before cannulation was compared with the value at increased shear stress after cannulation. However, Neal and Bates (33) could not find a positive correlation between imposed shear stress and \( L_p \) in microvessels of frog mesentery using a double cannulation method. It is not easy to directly compare our results with those of Neal and Bates (33), because the detailed time course data of \( J_s/S \) are not given. As shown in Fig. 3, the correlation between shear rate and \( L_p \) disappeared after 10 s of vascular occlusion, showing transient decline in the \( J_s/S \) response. This implies that measured \( J_s/S \) can differ depending on the time point of measurement. It is also possible that this discrepancy might result from the use of different species (frog vs. rat) and experimental technique (cannulated buffer-perfused vessel vs. autoperfused vessel).

Another question that remains unanswered is how quickly endothelial cells can respond to a change in shear. In general, it is known that certain mediators can alter microvascular permeability as quickly as seconds to minutes. Montermini et al. (32) observed rapid and sustained changes in permeability of small solutes within a few seconds of a change in perfusion rate, supporting our hypothesis that a step reduction in shear may account for the observed transient decrease in \( J_s/S \) after occlusion.

Inflammatory mediators can change microvascular permeability by activating various signaling pathways that lead to altered intercellular junctions by dissociation of junctional proteins and their connection to the cytoskeleton (42). It is well established that NO plays an important role in the regulation of microvascular permeability. Recent studies have demonstrated that NOS inhibitors can attenuate the acute increase in microvascular permeability due to various inflammatory mediators such as histamine, ATP, bradykinin, and platelet-activating factor (reviewed in Ref. 30). In acute inflammation, a calcium-activated, NO-cGMP-dependent pathway is believed to be a common pathway to increase microvascular permeability (13).

Regarding the role of shear stress in microvascular permeability, NO release by endothelial cells has been suggested to be an important mechanism (3). Investigations performed on individually perfused microvessels indicated that \( L_p \) is decreased by NOS inhibition (12, 35, 36). However, it also should be noted that the application of NOS inhibitors can induce an increase in leukocyte adhesion to venular endothelium and a subsequent increase in \( L_p \) in autoperfused capillaries when L-NAME (at a concentration of 100 \( \mu \)M) is treated for longer periods of time (20 min) (10). These findings suggest that the effect of NOS inhibition on \( L_p \) is dependent on the presence of neutrophils, with the effect reversed with anti-neutrophil serum or an antibody against the CD11/CD18 leukocyte adhesion molecule (10). Therefore, in the present study, the measurements with 50 \( \mu \)M L-NAME treatment were conducted within 5–15 min to attenuate the possible role of leukocyte adhesion in \( J_s/S \) measurements. Our in vivo observations of an attenuation in the \( L_p \) response with L-NAME in the presence of an increase in shear also support the previous in vitro findings (3, 37) that a change in fluid shear stress may regulate \( L_p \) by releasing NO.

Another interesting observation in the present study is that there was a differential sensitivity of \( L_p \) to shear rate between true capillaries and fourth-order arterioles. It would appear that capillary endothelium is more sensitive to a change in shear stress compared with endothelium of terminal arterioles (Fig. 7). The mechanisms responsible for this differential response in \( L_p \) remain unclear. However, based on the observation that NOS inhibition attenuated shear-induced change in \( L_p \) in capillaries, one possible explanation for this result is the differential expression of endothelial NOS (eNOS) in the rat mesenteric microcirculation. Kashiwagi et al. (20) observed abundant expression of eNOS in capillaries and venules in rat mesenteric tissue in vivo in contrast to little expression of eNOS in small arterioles.

**Sources of uncertainty in estimating normalized \( L_p \).** In estimating a normalized value of \( L_p \) with Eq. 9, the ratio of the pressure gradient is an important factor. In the current analysis, our previous measurements of an average value of arteriolar (A3) pressure were used in determining normalized \( L_p \). We used same-sized vessels with similar flow velocity in the same strain and age of rat (young Wistar rat, 200–250 g) in both the previous and current study to allow the use of previous pressure measurements in our current study. Considering a standard deviation of pressure measurements, baseline hydrostatic pressure of small arterioles was varied from 35 to 51 mmHg. In addition, the magnitude of step increases in pressure induced by arteriolar occlusion was varied from 5.4 to 9.4 mmHg, based on a standard deviation of 2 mmHg in actual pressure measurements of the pressure change. The mean arteriolar baseline pressure that we obtained (43 mmHg, Ref. 21) is virtually identical to that of similar-sized arterioles of rat intestinal muscle (45 mmHg, Ref. 9) and rabbit omentum (44 mmHg, Ref. 34). In addition, our measured 17% increase in pressure following occlusion is between the values reported in rabbit omentum (11% increase, Ref. 34) and hamster cheek pouch (22% increase, Ref. 29). In the present study, assumed values of osmotic reflection coefficients were used to estimate normalized \( L_p \) (\( \sigma_{baseline} = \sigma_{shear} = 0.9 \)). An alteration of osmotic reflection coefficient as well as \( L_p \) has been reported in the presence of inflammatory mediators. Thus we cannot rule out the possibility that our observed \( L_p \) at increased shear was overestimated due to the overestimation of \( \sigma_{shear} \).

To address these issues, we performed a sensitivity analysis by changing arteriolar pressure before and after arteriolar occlusion and osmotic reflection coefficient. The sensitivity of the normalized \( L_p \) to each variable can be seen in Fig. 10. For all cases, normalized \( L_p \) (shear vs. baseline) in the first seconds after occlusion was significantly different from one. This result indicates that uncertainties of pressure measurement and osmotic reflection coefficient might not be critical to our interpretations. Therefore, it is likely that a change in \( L_p \) is the major contributor to changes in \( J_s/S \).

In conclusion, the results of this study indicate the possible contributions of shear effect in regulating fluid filtration through endothelium after vascular occlusion in vivo. We have obtained evidence that an acute change in shear rate may regulate \( L_p \) of arteriolar and capillary endothelium. It appears that an acute change in shear regulates \( L_p \) in mesenteric capillaries via a NO-dependent mechanism. The differential response of \( L_p \) between arterioles and capillaries may indicate heterogeneity in endothelium sensitivity to a mechanical stimulus within a microvascular network. These findings support the idea that the endothelial transport barrier responds actively to changes in hemodynamic forces in the microcirculation and regulates transport pathways for water through biological as well as mechanical mechanisms.
REFERENCES