Microvascular blood flow resistance: role of endothelial surface layer

Axel R. Pries, Timothy W. Secomb, Helfried Jacobs, Markus Sperandio, Kurt Osterloh, and Peter Gaechgens. Microvascular blood flow resistance: role of endothelial surface layer. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2272–H2279, 1997.—Observations of blood flow in microvascular networks have shown that the resistance to blood flow is about twice that expected from studies using narrow glass tubes. The goal of the present study was to test the hypothesis that a macromolecular layer (glycocalyx) lining the endothelial surface contributes to blood flow resistance. Changes in flow resistance in microvascular networks of the rat mesentery were observed with microinfusion of enzymes targeted at oligosaccharide side chains in the glycocalyx. Infusion of heparinase resulted in a sustained decrease in estimated flow resistance of 14–21%, hydrodynamically equivalent to a uniform increase of vessel diameter by ~1 μm. Infusion of neuraminidase led to accumulation of platelets on the endothelium and doubled flow resistance. Additional experiments in untreated vascular networks in which microvascular blood flow was reduced by partial microocclusion of the feeding arteriole showed a substantial increase of flow resistance at low flow rates (average capillary flow velocities < 100 diameters/s). These observations indicate that the glycocalyx has significant hemodynamic relevance that may increase at low flow rates, possibly because of a shear-dependent variation in glycocalyx thickness.

rheology; microvascular networks; shear rate; glycocalyx

Several physiologically important functions of microvessels are known to be affected by the surface characteristics of microvascular endothelium. These characteristics are determined by a macromolecular lining, referred to as the glycocalyx, that includes both molecules directly bound to the plasma membrane of the endothelial cells and components adsorbed from the blood plasma. The glycocalyx has been shown to influence several aspects of vascular function, including the metabolic status of endothelial cells (31), endothelial permeability to water and solutes (32), leukocyte adhesion and emigration (11), and microvascular hematocrit (6).

The effect of the glycocalyx on capillary hematocrit has been attributed to a restriction of the luminal volume available for flow of plasma and red blood cells (6, 7). Such a restriction could also alter vascular flow resistance. Observations of blood flow in microvascular networks and direct measurements of pressure drop and volume flow rates in vivo have shown that the resistance to blood flow is about twice as large as expected from tube flow studies (12, 13, 23). A number of mechanisms that could increase flow resistance in vivo compared with that of glass tubes with corresponding diameters have been proposed: 1) irregularity of vessel lumen, 2) entrance effects at vascular branch points, 3) flow properties of white blood cells, and 4) restriction of flow by a macromolecular lining on the endothelial surface.

Available studies (4, 20, 28, 34, 35) suggest that the first three effects are not sufficient to quantitatively explain the observed high blood flow resistance in vivo. Therefore, the aim of the present study was to examine the effect of the glycocalyx on flow resistance by measuring changes in resistance after microinfusion of enzymes degrading components of glycocalyx oligosaccharide side chains. Because changes of surface shear forces caused by blood flow might affect glycocalyx properties, the effect of blood flow rate on flow resistance was also examined. The rat mesentery preparation used is well suited for these studies, because its microvessels do not exhibit spontaneous changes of vascular tone that might influence flow resistance during the experiments.

Materials and Methods

Intravital microscopy. Experiments were conducted in the microvasculature of the rat mesentery with intravital microscopy after approval of the procedures used by university and governmental committees on animal care. Details of the animal preparation and the setup used for intravital microscopy have been described elsewhere (22). Male Wistar rats (body wt 300–450 g) were prepared for intravital microscopy of the mesenteric microcirculation after premedication (atropine 0.1 mg/kg im and pentobarbital sodium 20 mg/kg im); anesthesia (ketamine 100 mg/kg im); cannulation of trachea, jugular vein, and carotid artery; and abdominal midline incision. During the experiments, which lasted for up to 2 h, the level of anesthesia and fluid balance were maintained by infusion of physiological saline (24 ml·kg⁻¹·h⁻¹ iv) containing 0.3 mg/ml pentobarbital sodium. Heart rate and arterial blood pressure (range 105–140 mmHg) were continuously monitored via the catheter in the carotid artery. The animals were transferred to a special stage mounted on an intravital microscope. The small bowel was exteriorized, and fat-free portions of the mesentery were selected for investigation with a ×25, NA 0.6 saltwater immersion objective (Leitz). In this preparation, vessels generally do not exhibit any spontaneous smooth muscle tone. As a precaution to prevent the development of tone and thus temporal variation of flow resistance during the measurement period, papaverine (10⁻⁴ M) was continuously superfused. Two series of experiments using different approaches were performed.

Bifurcation experiments. In a series of 28 experiments, changes in the flows in the two daughter branches of a bifurcating feeding arteriole were used to analyze the effect of enzymes and of buffer solutions with altered osmolarity microinfused into one daughter vessel and its dependent vessel network (Fig. 1). Changes of systemic hemodynamic
conditions, including blood pressure, upstream arterial vessel tone, and changes of blood rheological properties, can be assumed to have equivalent effects on the flows in both treated and untreated daughter vessels. Therefore, observed changes in the flow velocity ratio between these vessels reflect changes in the resistance of the treated vessel and its dependent network, independent of any systemic effects of the treatment.

Flow velocities in the daughter branches of the feeding vessel bifurcation (diameter \( \sim 30 \mu m \)) were measured using a dual-window method. Two rotatable pairs of photodiodes were mounted on micromanipulators and positioned in front of a screen onto which the microscopic image was projected (18). The photodiodes were aligned with the centerlines of the two daughter vessels. Given the overall magnification of the microscope system, the equivalent distance between the two photodiodes along the vessel axis was 5.5 \( \mu m \) in the mesenteric plane. The output signals of the photodiodes were amplified and sampled at a frequency of 10 kHz.

Recordings of 5-s duration were taken at intervals of \( \sim 0.5 \)–2 min and stored in a personal computer. These recordings were cross-correlated off-line using a Fourier transform algorithm to determine the temporal shift (delay) between the intensity patterns derived from each of the photodiode pairs. From the delay and the photodiode distance, the centerline flow velocity was determined. In independent calibration experiments using a rotating disk assembly, it was ascertained that this velocity measurement system yields reliable results up to 50 mm/s. This method provides a high time resolution (frequency of measurements \( \sim 100 \) Hz) and is well suited for measurement of relative changes in velocity in the two vessels, in the presence of rapid pulsatile fluctuations of velocity occurring simultaneously in both branches.

The percent change of resistance (\( \Delta R \)) in the treated network, fed by the microinfused daughter branch, was estimated from the changes in the flow velocity ratio between the two branches. Vessel diameters remained constant during the experiment as ascertained by occasional measurements. Flow velocity (\( V \)) in each branch is inversely related to the flow resistance (\( R \)) in that branch and its downstream ramifications. Therefore, the resistance of the network fed by the daughter branch to which the microinfusion was applied (\( R_T \)) is related, both before and after enzyme treatment, to the resistance of the untreated control network (\( R_U \)) by

\[
R_T / R_U = V_U / V_T
\]

Because \( R_U \) is assumed to be constant during the experimental procedure, \( R_T \) is proportional to the ratio of velocity in the untreated network (\( V_U \)) to velocity in the treated network (\( V_T \)). This ratio is not affected by changes in hemodynamic conditions upstream or downstream of the observed microvascular networks or by systemic rheological changes. If \( S_C \) represents the average value of \( V_U / V_T \) in the control state before treatment of one network and \( S \) represents a \( V_U / V_T \) value after treatment, \( \Delta R \) in the treated network can be expressed as

\[
\Delta R_T = 100 \cdot (S - S_C) / S_C
\]

Once the infusion micropipette was impaled into a side branch of one daughter vessel, approximately five velocity recordings of 5-s duration were made before microinfusion was started. One of the following substances was then infused for 7 min: vehicle solution (147 mM NaCl, 4 mM KCl, 3 mM CaCl\(_2\), 10 mM 3-(N-morpohino)propanesulfonic acid buffer, and 0.25 g/100 g bovine albumin (Sigma); \( n = 4 \)); vehicle solution in which osmolarity was altered by adjustment of NaCl concentration (50 mosmol/l (\( n = 3 \)), 800 mosmol/l (\( n = 1 \))); vehicle containing heparitin-sulfate lyase (10 U/ml, EC 3.2.1.18, Sigma); vehicle containing heparinase (100 U/ml, EC 4.2.2.8, Sigma; \( n = 4 \)); vehicle containing heparinase (100 U/ml, EC 4.2.2.7, Sigma; \( n = 6 \)); or vehicle containing neuraminidase (\( n = 2 \), 0.2 U/ml; \( n = 4 \), 5 U/ml; EC 3.2.1.18, Sigma). To avoid possible effects of high transmural pressure in the microinfused vessels, the perfusion pressure in the pipette was adjusted so that a small amount of blood (\( \sim 10\)–30% of the total flow rate) was still seen to flow in the arteriole in addition to the enzyme solution. As evidenced by direct pressure measurements in the network experiments (see below), the intravascular pressure increase elicited by microinfusion was \( <10 \) mmHg. After the infusion period,
velocity was recorded for up to 60 min. Sham experiments without microinfusion demonstrated that resistance ratios remained constant with time for similar observation periods.

Network experiments. To estimate resistance changes on heparinase treatment, 12 experiments were performed with this enzyme using a different technique. The flow resistance of microvascular networks in the rat mesentery was estimated by measuring arteriovenular pressure drop and volume flow rate before and after local microinfusion of heparinase (Fig. 1). The networks investigated were fed by arterioles with inner diameters of ~30 μm and drained by venules of ~45-μm diameter. As in previous studies in the same preparation, such networks consist of ~450 individual vessel segments (21). Intravascular pressures in the feeding arteriole and the draining venule were measured according to the servo-nulling technique using a micropipette with a tip diameter of ~1 μm connected to a micropressure measuring system (model 5, IPM; Ref. 9).

After an initial measurement of venular pressure, the micropipette was impaled into the main feeding arteriole where it remained during the experimental period (up to 60 min). For each determination of network flow resistance, arteriolar pressure was monitored and the image of the vessel, obtained with a charge-coupled device camera (MX, AIS), was recorded on videotape for ~30 s using asynchronous flash illumination (11360–1, Chadwick-Helmuth). The experiment was completed by a measurement of venular pressure to test the stability of arteriovenular pressure gradient and a measurement of zero pressure (in the superfusing solution) to test the stability of arteriolovenular pressure recording. The venular pressures at the beginning and the end of the experiment averaged 13.4 ± 1.8 and 11.8 ± 2.1 mmHg, respectively. A linear interpolation of the two venular pressures for each experiment was used in the calculations of flow resistance at a given time during the experiment. Because the micropipette remained in place before and after heparinase microinfusion, observed changes in resistance could not have resulted from perturbations of pressure caused by micropipette insertion. Furthermore, such perturbations were probably small, because the pipette tip was much smaller than the impaled vessel. These video recordings were analyzed off-line to determine arteriolar flow velocity and vessel diameter using a digital image analysis system (23). The spatial correlation analysis of recordings obtained with asynchronous illumination allowed measurements of blood flow velocities up to ~40 mm/s. A previously established method (23) based on parametric descriptions of the Fahraeus effect and a spatial averaging model was used to convert the obtained centerline velocities into mean blood flow velocities. A digital image analysis system was used to measure vessel diameters interactively (16). The precision of this method as determined by repeated measurements ranges between 0.5 and 1 μm (20). Because arteriolar diameters exhibited no detectable variations during the experiments or after enzyme treatment, relative changes of network flow resistance were calculated from relative changes in pressure drop and arteriolar flow velocity. In 9 of 12 experiments of this series, the network was perfused for 5 or 10 min with heparinase in the vehicle solution described above via a micropipette impaled into the feeding arteriole. Velocity data and arteriolar pressure readings were sampled several times in the control state, as well as repeatedly for ~30 min after the end of the heparinase infusion, to allow estimations of network flow resistance.

In three additional experiments of this series, the influence of variation of flow rate on network flow resistance was analyzed without any previous enzyme treatment. A graded reduction of blood flow in the microvessel networks was achieved by partial occlusion of the feeding arteriole with a blunted micropipette proximal to the point of pressure measurement. Graded flow reduction was repeated after varying the systemic hematocrit (Hs) to different levels between 0.52 and 0.07 by isovolemic or hypervolemic exchange of blood with homologous red blood cells, plasma, or hydroxyethyl starch solution (100 g/l, mol wt 200,000/0.5 in physiological saline where 0.5 indicates the degree of substitution in the starch molecule; Fresenius) according to a procedure described previously (23).

Because reduction of flow rate in the feeding arteriole by partial occlusion may lead to upstream phase separation and thereby decrease hematocrit and flow resistance in the network studied, the hematocrit in the feeding arteriole was continuously measured by off-line videodensitometric analysis (17, 22). The image of the vessel was recorded on videotape using monochromatic illumination (wavelength 448 nm). An in vivo calibration for each vessel was established, assuming that the discharge hematocrit in the arteriole (H) in the unoccluded state was equal to Hs. Experimental values of optical density (OD) measured in the unoccluded vessel during variation of Hs were used to determine the parameters a and b of the linear regression

\[ \text{OD} = a + b (2Hs + H)^3 \]

With this calibration equation, H was calculated from the measurements of OD obtained during the occlusion procedures, when H was expected to be lower than Hs. The effect of changes in H on network flow resistance R was determined using the equation

\[ R = A + B \cdot [ (1 - Hs)^3 - 1 ] - B \cdot [ (1 - H)^3 - 1 ] \]

The empirical parameters A, B, and C were determined by a fitting procedure using the set of experimental data on network flow resistance R for different levels of Hs in the unoccluded state. An equation of this form was used previously to adequately describe the relation between flow resistance and hematocrit in tube flow (19). The resistance measurements made during partial occlusion (R0) were then corrected (Rc) for the measured changes in hematocrit according to the equation

\[ R_c = R_0 \cdot \frac{A + B \cdot [(1 - Hs)^3 - 1]}{A + B \cdot [(1 - H)^3 - 1]} \]

Statistics. Mean values were compared by one-way analysis of variance procedure using Tukey-B correction for post hoc multiple comparisons. Significance is indicated at the P < 0.05 level.

RESULTS

Table 1 gives averaged morphological and hemodynamic values for arterioles of both bifurcation and network experiments. In the bifurcation experiments, vessel diameters were measured at the site of velocity measurement. In these experiments, no statistically significant difference between the parameters for the two branches (infused and untreated) of the observed bifurcation was observed.

Figure 2 shows results of heparinase infusion in a typical bifurcation experiment. Flow velocity in the untreated branch exhibited no substantial change during the experimental period, whereas flow velocity in the treated branch increased substantially after the
enzyme infusion. This indicates a reduction of flow resistance in the microvascular network fed by the treated branch. Average changes of flow resistance with time after vehicle infusion and heparinase infusion in the bifurcation experiments are shown in Fig. 3. Postinfusion values of flow resistance change are slightly elevated after infusion of the vehicle (n = 4 experiments), but this is statistically significant only for the first 5 min of the postinfusion period. In contrast, flow resistance in the heparinase infusion experiments (n = 6) exhibited a significant and sustained decrease during the observation period that ranged from 30 to 60 min in individual experiments.

Microinfusion of neuraminidase into one daughter branch of arteriolar bifurcations led to a significant elevation of flow resistance of the treated microvessel network already in the first measurement after the end of the microinfusion and a further progressive increase with time (Fig. 4). Intravital microscopic inspection showed that this resistance increase was accompanied by adherence and rolling of large numbers of platelets on the endothelial surface in all vessel branches downstream of the infusion site.

### Table 1. Mean values and standard deviations of morphological and hemodynamic parameters for arterioles feeding networks studied

<table>
<thead>
<tr>
<th></th>
<th>Bifurcation experiments (n = 28)</th>
<th>Network experiments (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter, µm</td>
<td>Mean 20.3 ± 3.6</td>
<td>Mean 29.6 ± 5.4</td>
</tr>
<tr>
<td>Pressure, mmHg</td>
<td>NM ± NM</td>
<td>64.1 ± 15.3</td>
</tr>
<tr>
<td>ΔP, mmHg</td>
<td>6.7 ± 2.1</td>
<td>52.2 ± 15.5</td>
</tr>
<tr>
<td>Flow Velocity, mm/s</td>
<td>144 ± 98</td>
<td>16.2 ± 7.2</td>
</tr>
<tr>
<td>Volume Flow, nl/min</td>
<td>330 ± 188</td>
<td>740 ± 412</td>
</tr>
<tr>
<td>Shear Rate, diam/s</td>
<td>6 ± 98</td>
<td>546 ± 216</td>
</tr>
</tbody>
</table>

ΔP, pressure drop from feeding arteriole to draining venule; diam, vessel diameters; NM, not measured; n, no. of experiments.

**Fig. 3.** Changes in network flow resistance over time after 7-min microinfusion of vehicle (n = 4 experiments) and heparinase (100 U/ml, n = 6 experiments). Values from individual bifurcation experiments were averaged for successive 5-min time intervals and are shown with standard errors. For observation times exceeding 35 min, data were averaged over 10 and 15 min.

**Fig. 4.** Changes in network flow resistance over time on infusion of neuraminidase (5 U/ml, n = 4 experiments) in bifurcation experiments. Data representation as in Fig. 3.
Figure 5 compares absolute values of flow resistance obtained in the network experiments for the untreated state with those seen after microinfusion of heparinase. Although in all but one experiment a significant reduction of resistance was seen after 5 min of infusion, only a small additional effect was seen after 10 min.

To summarize the results of both experimental designs, changes in flow resistance on treatment were averaged for each individual experiment over the postinfusion observation period. Mean values (± SD) of these averages are compared in Fig. 6 for the different treatment groups. The infusion of vehicle as well as of buffer solutions with altered osmolarity (bifurcation experiments) led to small but not significant increases of flow resistance. No change in resistance was observed after injection of heparitin-sulfate lyase (bifurcation experiments). In contrast, heparinase treatment resulted in a substantial reduction of flow resistance in both the network experiments (mean reduction $-20.8 ± 8.5\%$, 10-min infusion; $-16 ± 13.4\%$, 5-min infusion) and the bifurcation experiments ($-14.2 ± 6.5\%$, 7-min infusion). These changes were statistically significant. The average resistance increase for the first 15 min after the end of the neuraminidase infusion was $98.5 ± 25\%$ for the higher enzyme concentration tested (bifurcation experiments).

The effect of alterations in blood flow rate by partial microocclusion of the feed vessel on network flow resistance is shown in Fig. 7. Measured flow velocities in the feeding arteriole were converted into pseudo-shear rates ($\lambda$) by dividing by vessel diameter. If it is assumed that measured velocities represent means over vessel cross sections and that velocity profiles are parabolic, pseudo-shear rates may be converted to wall shear rates by multiplying by 8. Because these assump-
tions may not be valid, the use of $U$ values is preferable. At pseudo-shear rates in the feeding vessel below \( \sim 100 \) diameters/s, flow resistance increased to about twice the value measured at high shear rates. This increase was seen both in the raw data (not shown) and in data corrected for the hematocrit reduction occurring during the occlusion procedure (Fig. 7). Data obtained over a large range of systemic hematocrit (0.07–0.52) exhibit very similar trends. This suggests that $H_5$ does not substantially influence the increase in network flow resistance at low levels of $U$.

**DISCUSSION**

The key finding of the present study is that heparinase microinfusion led to a sustained 14–21% decrease in flow resistance of microvascular networks downstream of the site of infusion. Such an effect was not found when enzyme-free vehicle or solutions of altered osmolarity were infused. If endothelial swelling or shrinking occurs during hypo- or hyperosmolar microperfusion, this effect is apparently rapidly reversed on reperfusion with normal blood and thus not detectable in the present experimental design. Heparitin-sulfate lyase led to no measurable decrease of flow resistance. After neuraminidase, a doubling in flow resistance was observed, consistent with the results of Mühliesen et al. (14) for the isolated perfused ileum. In the present experiments, microscopic observation showed the presence of a layer adjacent to the endothelium composed mainly of adherent and rolling thrombocytes.

**Glycocalyx thickness.** Changes in resistance imply changes either in the effective vessel diameter or the apparent blood viscosity. Because the amount of solution microinfused was very small (between 1 and 7 µl), the systemic concentration of the enzymes applied is probably negligible, and it can be assumed that the rheological properties of blood entering the experimental site on terminating the microinfusion were unaffected by the preceding treatment. In addition, the results obtained in the bifurcation experiments are independent of changes of systemic hemodynamic conditions including blood viscosity. Therefore, the observed changes in flow resistance most likely reflect changes in effective vessel diameter. The vessels investigated were fully dilated, and no pressure changes were seen after the end of microinfusion. Therefore, changes in morphological vessel diameter are unlikely. However, the decrease in network flow resistance after heparinase treatment could result from reduction of the thickness of the glycocalyx, leading to an increase of the effective vessel diameter available for red blood cell and plasma motion.

The increase in effective vessel diameter that would be required to produce the observed decrease in resistance may be estimated using a theoretical simulation of flow in microvascular networks (22). This simulation uses geometries (diameters and lengths of the vessels, network topologies) determined previously (22) for networks in the rat mesentery of a size similar to the networks studied in the present study and assumes a relationship between apparent blood viscosity, vessel diameter, and hematocrit deduced from studies of blood flow in mesenteric networks (23).

According to this simulation, a uniform increase of effective radius in all perfused microvessels of \( \sim 0.35 \) µm could account for the observed reduction of flow resistance in the bifurcation experiments on microinfusion of heparinase. For the network experiments, a value of \( \sim 0.55 \) µm is obtained. These values represent lower bounds on the thickness of the layer present on the endothelial surface in the untreated state. Equivalent reductions in resistance could also result from a nonuniformly distributed increase in diameter. In that case, the increase in effective radius would be larger than the above estimates in some parts of the networks and smaller in others. Because, for the purpose of this estimation, the glycocalyx is considered as a stiff, impermeable layer of uniform thickness lining all vessels in the network, the assumption of a permeable glycocalyx, permitting axial plasma flow (5), would increase these estimates. Also, the removal of the glycocalyx by the heparinase treatment was probably incomplete, because heparinase acts at specific sites within the oligosaccharide side chains of glycoproteins residing on the endothelial surface and does not cleave other (nonsulfated) sugar components or the protein backbone of these molecules.

The above estimates of glycocalyx thickness are consistent with results of previous studies. On the basis of measurements of capillary hematocrit in resting striated muscle, Duling and Desjardins (7) suggested that plasma flow is retarded in a layer on the endothelial surface, the thickness of which was estimated between 0.8 and 1.8 µm. Further studies (6) showed that treatment with heparinase caused a persistent increase in capillary hematocrit, indicating a reduction in the thickness of the layer. Using a different approach, Vink and Duling (33) observed capillaries in the hamster cremaster muscle and demonstrated a layer of 0.4–0.5 µm adjacent to the endothelial cells that was inaccessible to flowing red blood cells and plasma.

In contrast, the apparent glycocalyx thickness seen in electron microscopic studies is \( \sim 0.1 \) µm (8, 15). However, electron microscopy requires fixation and staining procedures that may cause collapse of the glycocalyx proteoglycans (6). Indirect methods have led to higher estimates of layer thickness. From measurements of the sialic acid concentration on the surface of vascular endothelium (2), Silberberg (29) estimated a thickness of the glycocalyx gel under native conditions of at least 0.5 µm and noted that this gel would collapse to \( \sim 0.05 \) µm if dehydrated during preparation for electron microscopy.

**Possible contribution of plasma proteins to glycocalyx properties.** Endothelial cells bind plasma proteins, including fibrinogen and albumin (26, 37). Observations of endothelium-coated beads sedimenting in different media (24) imply that plasma proteins affect fluid flow past endothelial cells. Surprising reductions of falling velocities from expected values were found in plasma but not in other media. Based on Stokes’ law for the
drag on a sphere, the reduction in sedimentation velocity in plasma can be interpreted as an increase in the effective radius of the beads by ~25 µm. Formation of a layer of this thickness capable of impeding flow may depend on the low fluid shear rate on the surface of the falling beads (<3.5 s⁻¹) compared with average wall shear rates in the microvessels in the mesentery (~200–2,000 s⁻¹; Ref. 21).

These observations indicate that plasma protein levels may influence the mechanical properties of the glycocalyx and suggest a possible origin for its stiffness. Adsorption of plasma proteins to other glycocalyx components may generate a colloid osmotic pressure exceeding that of free plasma (36) if the plasma protein molecules are loosely bound and retain significant mobility. For mechanical equilibrium, such an increase of colloid osmotic pressure must be balanced by tension in the membrane-bound glycoproteins (30). To compress the glycocalyx, an applied force must overcome this pressure increment. The pressure exerted by the rim of a substantially deformed red blood cell is of the order k/L, where k is the shear elastic modulus of the membrane and L is a characteristic length scale. Taking k = 0.006 dyn/cm and L = 1 µm yields 60 dyn/cm²; a similar estimate is implied by more detailed pressure calculations (27). An increase in colloid osmotic pressure within the glycocalyx by only 1% (i.e., 330 dyn/cm², based on a colloid osmotic pressure of 25 mmHg) could thus generate sufficient force to resist penetration by red blood cells. The finding that short-term microinfusion of artificial plasmas with altered protein content had little effect on capillary hematocrit (10) may indicate that equilibration of proteins between plasma and glycocalyx exhibits time constants above ~10 min.

Effect of flow rate on flow resistance. When blood flow rate was reduced by graded microocclusion, network flow resistance remained approximately constant at U levels down to ~100 s⁻¹ in the feeding arterioles (Fig. 7). However, flow resistance increased at flow rates below this level. The resistance increase at low shear rates was similar for all levels of systemic hematocrit (range 0.07–0.52) and seems therefore to be independent of red blood cell concentration.

Several mechanisms for this behavior may be proposed. 1) In bulk shear flow, blood exhibits strong shear-thinning behavior. However, flow resistance in narrow glass tubes (diameter ~30 µm) perfused with red blood cells suspended in plasma shows little or no change of flow rate with U in the range from 1 to 100 s⁻¹ (1, 25). Because average U values in venules with diameters >20 µm are about one-eighth of those in the feeding arteriole (21), the threshold U level of 100 s⁻¹ in the feeding arteriole corresponds to ~12 s⁻¹ in larger venules. Therefore, the observed increase in resistance is not accounted for by the rheological behavior of blood in narrow uniform tubes. 2) The observed increase of resistance on microocclusion could result from a passive diameter change caused by the reduction of capillary pressure. Bosman et al. (3) showed passive changes in capillary diameter by ~6% and +12% during aortic occlusion causing a fall of capillary pressure and reactive hyperemia causing an elevation of capillary pressure. Such diameter changes are too small to explain the resistance increase of up to 100% observed in the present experiments with decreasing shear rate. 3) The thickness of the glycocalyx may increase at very low flow rates (U < 100 s⁻¹). Desjardins and Duling (6) reported that capillary hematocrit in muscle capillaries increased with flow velocity from ~0.18 to 0.27 mm/s. Capillary velocities in this range correspond to those at which flow-dependent resistance was evident in the present study. This last mechanism is therefore consistent with observed flow dependence of flow resistance observed here.

Discrepancy between in vivo and in vitro estimates of network flow resistance. Measured values of flow resistance in microvascular networks have been found to be about twice as large as expected from measurements of flow resistance in glass tubes (23). Flow restriction by the glycocalyx is a possible reason for this difference. The relation between glycocalyx thickness and network flow resistance can be estimated using the theoretical simulation already described. According to this simulation, a uniform, impermeable glycocalyx of 1.5-µm thickness would produce the difference in flow resistance of microvascular networks of the rat mesentery compared with predictions based on glass tube rheology.

In reality, however, other effects may also contribute to the increased flow resistance in vivo. The hydrodynamic resistance of a vessel with irregular luminal contour is larger than the resistance of a uniform tube with the same mean diameter (20) because contour irregularity leads to additional energy dissipation caused by transient deformation of red blood cells (28). Additional flow resistance may occur at vascular branch points (28). Available estimates of these effects suggest that they may cause a reduction of effective vessel radius by not more than ~0.5 µm. The effect of white blood cells on flow resistance of vascular beds has been debated, and reported resistance increases caused by white blood cells in skeletal muscle range from 1 to 20% (4, 34, 35). In the mesentery, capillaries are wider than in skeletal muscle and effects of white blood cells on resistance can be expected to be lower. Thus the observed difference between flow resistance in vivo and in vitro is only partly explained by effects of vascular irregularity, entrance phenomena, and white blood cells. The remaining discrepancy can, however, be accounted for by the presence of a stiff, impermeable layer with thickness of >0.5 µm adjacent to the endothelium.

In conclusion, the present findings support the concept of a macromolecular layer (glycocalyx) at the luminal surface of microvascular endothelium that contributes significantly to microvascular flow resistance. This layer may have significant implications for the many physiological functions in which endothelial cells are involved. Such functions could therefore be affected by changes in plasma protein composition that modify the composition and thus relevant properties of the endothelial glycocalyx.
REFERENCES


