Redistribution of Red Blood Cell Flow in Microcirculatory Networks by Hemodilution

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The effect of isovolemic hemodilution on red blood cell flow distribution was studied in complete self-contained microvessel networks of the rat mesentery. Hematocrit, diameter, and length of all vessel segments as well as the topological structure were determined in control networks (systemic hematocrit, 0.54) and after hemodilution (systemic hematocrit, 0.30). Hemodilution was performed by exchanging blood with hydroxyethyl starch (MW 450,000; 6%) or homologous plasma. With hemodilution, the decrease of microvessel hematocrit exceeded that of systemic hematocrit. The average discharge hematocrit in capillaries was 79% of systemic hematocrit in the control group and 73% with hemodilution (p<0.001). The heterogeneity of capillary hematocrit within the network, expressed by the coefficient of variation, increased from 0.4 to 0.7. By using the morphological and topological data of four networks, the distribution of hematocrits was also calculated using a hydrodynamic flow model. The modeling results were found to be in close agreement with the experimental data. This indicates that the observed changes can be deduced from established rheological phenomena, most of all phase separation at arteriolar bifurcations. The changes in hematocrit distribution after hemodilution are accompanied by a redistribution of red blood cell flow within the network: relative to total red blood cell flow, red blood cell flow in the distal capillaries of the network increases by about 40% at the expense of the proximal capillaries that are close to the feeding arteriole and that exhibit the highest red blood cell flow under control conditions. This redistribution leads to a more homogeneous red blood cell flow distribution between proximal and distal regions of vessel networks with hemodilution, which might serve to increase tissue oxygenation in regions endangered by underperfusion. (Circulation Research 1992;70:1113–1121)

Key Words • Hemodilution • Microcirculation • Networks • Flow Pathways • Hematocrit

I ntentional hemodilution has evolved as a clinically accepted therapy of a number of disorders with impaired peripheral perfusion. The beneficial effects reported have been attributed to increased peripheral perfusion caused by reduced blood viscosity and possibly peripheral vasodilation. The present study will mainly focus on effects resulting from changes in the rheological properties of the blood after hemodilution, which will affect the perfusion characteristics of all tissues both in the presence and absence of vascular reactions.

At a given driving pressure and oxygen saturation of hemoglobin, oxygen delivery to a tissue is proportional to the hematocrit of the systemic blood divided by the flow resistance. The latter, in turn, is the product of geometric hindrance of the vascular bed and blood viscosity. Because of the dependence of blood viscosity on hematocrit, oxygen delivery or red blood cell flow to a vascular network is a nonlinear function of hematocrit with a maximum at the so-called optimal hematocrit. There is considerable variation in the determined values of the optimal hematocrit: in experiments in which changes of vessel diameters are unlikely (e.g., in maximal dilatation), the optimal hematocrit was reported to be in the range of 0.5,2,3 whereas in tissues in which vasodilatory reserve was available, values in the range of 0.3 were found.4–6 The latter findings have served as a rationale to advocate intentional hemodilution as a means to alleviate tissue malperfusion in peripheral ischemic disease.1 Because it is not clear whether insufficiently perfused tissue areas representing the target zones of intentional hemodilution retain vasodilatory capacity, it appears uncertain whether the concept of optimal hematocrit is adequate to explain the positive effects of hemodilution.

Hemodilution not only changes oxygen-carrying capacity and viscosity of the blood, but also influences the distribution of hematocrit and red blood cell flows within microvascular networks. Under physiological conditions, the volume concentration of red blood cells in microvessels (Hv) is much lower than the systemic hematocrit (Hs):7–12 In addition to a low mean value, the Hv values show a substantial heterogeneity.9–11 It has been suggested in some studies that in hemodilution the average capillary Hv falls less than Hs, and red blood cells are distributed more homogeneously to the individual microvessels.13–18 Both effects are supposed to improve tissue oxygenation. However, all of the cited studies are based on hematocrit measurements in selected subpopulations of microvessels. In view of the large heterogeneity of flow and hematocrit distribution characterizing microvascular networks,9–11,19,20 it is very difficult to
select a group of vessels that is truly representative of the entire vascular bed. Even if such selection of vessels were possible in the control state, during hemodilution redistribution of cell and plasma flows within the network would invalidate the chosen set of selection criteria.

The present study attempts to overcome these problems by determining hematocrit, length, diameter, and topological position of all vessel segments in complete networks of the terminal vascular bed comprising large numbers of flow pathways from arterioles to venules. The hematocrit data are compared with predictions of a hydrodynamic flow model\(^{22}\) that is based on established rheological data on blood viscosity and phase separation at bifurcations. These model calculations also enable the analysis of red blood cell flow distribution and thus oxygen delivery.

The rat mesenteric microcirculation was chosen for this study because most of the precapillary microvessels lack smooth muscle cells.\(^ {23} \) Moreover, spontaneous changes of vasomotor tone are not observed. Therefore, the changes in perfusion pattern determined in this vascular bed mainly reflect changes of blood rheology that are operative in all tissues and add to effects exerted by organ-specific active control mechanisms.

**Materials and Methods**

**Animal Experiments**

Male Sprague-Dawley rats (body mass, 300–460 g) were anesthetized with ketamine (100 mg/kg i.m.) after premedication with atropine (0.1 mg/kg i.m.) and pentobarbital sodium (20 mg/kg i.m.). The trachea, left carotid artery, and jugular vein were cannulated with polyethylene tubing. Throughout the experiment, all animals received an intravenous infusion (24 ml·kg\(^{-1} \cdot \)hr\(^{-1} \)) of physiological saline containing 0.3 mg/ml pentobarbital sodium to maintain anesthesia and prevent an increase of \(H_{sv} \). Arterial blood pressure, heart rate, and central venous pressure were monitored continuously. \(H_{sv} \) (heparinized microhematocrit tubes) and red blood cell counts (Coulter Dn, Herts, UK) were determined before and after hemodilution steps and at approximately 30-minute intervals during intravital microscopy in blood samples drawn from the carotid catheter.

Animals were allocated to a control (CTRL, \(n=5\)) and two hemodilution (DIL, \(n=6\)) groups. Isovolemic hemodilution was performed either with homologous plasma (PLASMA group, \(n=3\)) or with hydroxyethyl starch solution (60 g/l, MW 450,000/0.7 in physiological saline; Fresenius, Wiesbaden, FRG; HES group, \(n=3\)). Homologous plasma was obtained by bleeding an anesthetized donor rat through the carotid catheter and centrifuging the blood at 3,000g. Hemodilution was performed in two steps by exchanging 10% (2.4–3.3 ml) and then 11.5% (2.7–3.8 ml) of estimated blood volume (8% of body mass) in the PLASMA group, and 11% (3.4–3.8 ml) and 13% (4.0–4.5 ml) of estimated blood volume in the HES group, respectively. Each hemodilution step was performed by simultaneously withdrawing (from the carotid catheter) and infusing (through the jugular catheter) the specified volumes in approximately 10–15 minutes with a 15-minute interval between dilution steps. The resulting \(H_{sv} \) after hemodilution ranged between 0.30 and 0.35 in the PLASMA group and between 0.29 and 0.325 in the HES group. The CTRL group remained untreated and showed values of \(H_{sv} \) between 0.48 and 0.51. Mean red blood cell volume in the carotid blood, as calculated from red blood cell concentration and hematocrit, was 54±2 fl in the CTRL group as well as in the PLASMA group, while the HES group exhibited mean red blood cell volume values of 48±1 fl.

For intravital microscopy, the mesentery was cautiously exteriorized through an abdominal midline incision, spread over a Lucite stage, and superfused with thermostated (37°C) bicarbonate-buffered saline (composition, millimoles per liter: NaCl 132, KCl 4.7, CaCl\(_2\) 2.2, MgCl\(_2\) 2.1, and NaHCO\(_3\) 18, equilibrated with 5% CO\(_2\) in N\(_2\), pH 7.3–7.4). Microscopy was performed using a Leitz intravital microscope modified for telescopic imaging\(^ {23}\) and equipped with a Leitz SW 25/0.6 saltwater immersion objective, a projection eyepiece (f=268 mm, Leitz) and a transfer lens (f=300 mm, Xenar, Schneider, Kreuznach, FRG), and a video camera (model 1005, RCA, Lancaster, Pa.). The mesenteric microcirculation was transilluminated with monochromatic blue light (BG12 color glass and MA 3-0.3, 448-nm small band interference filter; Schott, Mainz, FRG). Simultaneous recordings were obtained on videotape (Sony U-matic) and on black-and-white film (AGFAPAN 100 professional, Agfa, Leverkusen, FRG). The final magnification on the target of the video camera and the film plane of the photographic camera was ×28, yielding a field of view of approximately 300×400 μm on the video monitor. Scanning microvessel networks in the fat-free portion of the mesentery (area between 25 and 80 mm\(^2\)) took approximately 30 minutes and resulted in up to 300 photographs that were assembled into photomontages.

**Data Analysis**

The number of vessel segments (defined as the section of a vessel between two branch points) per network ranged between 334 and 913 in the CTRL group, between 431 and 550 in the PLASMA group, and between 328 and 456 in the HES group. Segment diameters and lengths were measured from photomicrographs. Flow direction and microvessel hematocrit were obtained from video recordings.\(^ {9,24} \) The method of photometric hematocrit measurement used is based on the determination of optical density in the vessel segment at an isosbestic wavelength of hemoglobin (448 nm) by using a digital video image analysis system.\(^ {25} \) Calibration of the method with capillary glass tubes perfused with blood of known hematocrit allowed determination of both microvessel hematocrit (tube hematocrit, \(H_T \)) and discharge hematocrit (\(H_D \)), assuming that the Fahraeus effect in vivo is equal to that in the calibration tubes. In the HES group, a correction for the observed change in mean red blood cell volume was applied. In most (85%) of the vessels with diameters below 8 μm, \(H_T \) was determined from photomicrographs by counting the number of red blood cells in a given length of the vessel. \(H_T \) was then derived from \(H_T \) by using literature data of the Fahraeus effect in vitro.\(^ {21} \) In these smallest vessels the photometric method was used only if \(H_T \) was too high to allow cell counting.
Vascular networks were divided into arteriolar and venular vessel trees on the basis of flow directions in individual vessel segments. Segments connecting two divergent branch points and linked to an input vessel of the network via diverging branch points were classified as arteriolar. A corresponding definition using converging branch points defined the venular segments. Segments connecting the divergent arteriolar tree with the convergent venular tree (arteriovenous segments) were classified as “capillaries” regardless of their diameter or other morphological criteria. This definition of capillaries is purely topological and therefore includes vessel segments whose morphological and hydrodynamic properties vary. The mean capillary diameter of all networks analyzed in this study was 9.6±2.5 μm, whereas individual values ranged from 4.5 to 21 μm. When capillaries were seen to form meshes creating divergent bifurcations downstream of a convergent bifurcation, only the initial capillary branch connected to the arteriolar tree was included in the analysis; less than 1% of all capillaries was seen to form such meshes. One network of the CTRL group and two networks of the HES group consisted of two arteriolar trees that could be analyzed independently. All other networks were fed by one large inflow arteriole only.

The topological position of each microvascular segment in the arteriolar tree is represented by its generation number, which equals the number of branch points between this segment and the inflow arteriole of the vessel tree plus one. Based on this generation scheme, consecutive complete flow cross sections were defined through which the blood entering a vessel tree must pass: the complete flow cross section number “x” consists of all arteriolar and capillary segments of generation x plus the capillaries of lower generation numbers. Depending on the maximum generation number reached in a tree, this type of analysis yields about 20 complete flow cross sections, the most distal flow cross section being identical to the population of capillaries.

Model Calculations

A hydrodynamic model simulation was used to calculate the distribution of red blood cell and plasma flow in four microvascular networks (two networks from the CTRL group, one network each from the PLASMA and HES groups). The model calculations are based on the individual morphology and topology of each network and the rheological laws derived from literature data.

The network data bases list diameter, length, and individual feeding or draining segments for all segments in a microvascular network. For segments entering or leaving the network, measured hematocrit and estimated volume flow values were used as boundary conditions. For each network analyzed with the model, inflow hematocrit could be manipulated to match the experimental values of both DIL and CTRL groups. The volume flow in the main arteriolar inflow vessel was set to correspond to measurements reported in the literature for vessels of the appropriate dimensions. For additional input or output segments, the volume flow values were calculated based on the volume flow in the main arteriolar inflow vessel in proportion to the number of capillaries fed by or drained by the respective inflow or outflow vessel.

Rheological phenomena represented in the hydrodynamic model were the phase separation effect at arteriolar branch points and the variation of apparent blood viscosity with hematocrit and vessel diameter (Fahrneus-Lindqvist effect). For the nonproportional distribution of red blood cells and plasma at arteriolar branch points (phase separation), a parametric description was used that is based on data obtained from arteriolar bifurcations in the rat mesentery in previous studies. According to these data, the hematocrit difference between the daughter vessels of a bifurcation is determined by the diameter and hematocrit of the feeding vessel, the relation between the diameters of the daughter vessels, and the flow split at the bifurcation.

An equation similar to that given by Pries et al was used to estimate the relative apparent viscosity of blood in a microvessel (ηrel) as a function of HD and vessel diameter (D, in micrometers). This equation was fitted to experimental data available in the literature

\[ \eta_{rel} = \left(1 + \frac{H_D}{0.45} \cdot L^{-1}\right) \cdot (220e^{-1.543D} + 2.17 - 2.45e^{-0.071D}) \cdot \left(\frac{D}{D-W}\right)^4 \]

with

\[ \alpha = 3.9(1+e^{-2.374\cdot(D-5.054)}) \]

W is a correction factor that was previously shown to optimize the agreement between model results and experimental data. In the present study, W ranged between 2.4 and 3.4 μm.

Results

Hemodilution reduced mean hematocrit in the capillaries (segments connecting the arteriolar with the venular vessel trees) to a similar extent in the HES and PLASMA groups (Table 1). Average capillary Hr in the HES and PLASMA groups decreased more than Hsys, i.e., the ratios Hr/Hsys and Hr/Hsys decreased. The dispersion of capillary Hr/Hsys and Hr/Hsys increased as reflected by an elevation of the standard deviations and coefficients of variation. The distributions of Hr/Hsys and Hr/Hsys in the dilution groups (PLASMA, HES, and DIL) were tested against that of the CTRL group by using the nonparametric U test (Wilcoxon-Mann-Whitney), which is mainly sensitive to differences of the median. These tests indicated statistically significant differences (p<0.001). In contrast, no significant difference was found between the PLASMA and HES groups (p=0.66 and p=0.89).

Both for the experimental and for the model results, the hematocrit distributions obtained after hemodilution were flatter and exhibited lower mean values compared with those of the CTRL group (Figure 1). Because the differences between the distributions obtained in the PLASMA and HES groups were small and the medians showed no statistically significant difference, the results of experimental hemodilution will be given for the combined DIL group in the following. Figure 1 demonstrates that the model results exhibit a
higher number of capillaries in the lowest hematocrit classes. This is due to the prediction of significant numbers of segments with zero hematocrit by the model. Such capillaries are presumably underrepresented in the experimental results because they are difficult to detect in the analysis of photomontages or video recordings obtained during the experiments.

Figure 2 shows hematocrit distributions for the subgroup of capillaries with diameters less than 8 μm, most of which (approximately 85%) were analyzed by direct cell counting. In agreement with the findings for the complete capillary group, the hematocrit distribution exhibits lower median values and higher coefficients of variation after hemodilution compared with control conditions.

Figure 3 shows the average H₀, normalized with respect to the inflow hematocrit of the networks for the consecutive flow cross sections of arteriolar vessel trees from the inflow arteriole to the level of the capillaries. In the averaging procedure, the H₀ values were weighted by the individual cross-sectional area of the vessel segments. Since by definition total blood flow through the tree passes through each of the consecutive complete flow cross sections, a flow-weighted average H₀ always equals the inflow hematocrit. If, on average, those vessel segments of a complete flow cross section that exhibit high flow velocities also exhibit high H₀ values (such vessels may correspond to functional red blood cell shunts as described in the literature), red blood cells will travel faster through that cross section than will plasma. As a consequence, the mean area-weighted H₀ will be lower than the inflow hematocrit of the vessel tree. This effect has been called the network Fahraeus effect. As shown in Figure 3, the hematocrit reduction caused by the network Fahraeus effect in-

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**TABLE 1. Statistical Parameters for Capillary Hematocrits**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of networks</th>
<th>No. of arteriolar trees</th>
<th>No. of capillaries</th>
<th>H₀ median</th>
<th>H₀/H₀sys median</th>
<th>CV</th>
<th>H₀ median</th>
<th>H₀/H₀sys median</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL</td>
<td>5</td>
<td>6</td>
<td>801</td>
<td>0.23</td>
<td>0.48</td>
<td>0.45</td>
<td>0.37</td>
<td>0.78</td>
<td>0.40</td>
</tr>
<tr>
<td>PLASMA</td>
<td>3</td>
<td>5</td>
<td>491</td>
<td>0.12</td>
<td>0.37</td>
<td>0.88</td>
<td>0.20</td>
<td>0.63</td>
<td>0.75</td>
</tr>
<tr>
<td>HES</td>
<td>3</td>
<td>5</td>
<td>308</td>
<td>0.13</td>
<td>0.40</td>
<td>0.61</td>
<td>0.21</td>
<td>0.67</td>
<td>0.58</td>
</tr>
<tr>
<td>DIL</td>
<td>6</td>
<td>8</td>
<td>799</td>
<td>0.12</td>
<td>0.39</td>
<td>0.70</td>
<td>0.21</td>
<td>0.65</td>
<td>0.70</td>
</tr>
</tbody>
</table>

CV values are valid for the normalized hematocrit values (H₀/H₀sys, H₀/H₀sys), but the corresponding values for H₀ and H₀sys differ by less than 2%. H₀, tube hematocrit; H₀/H₀sys, H₀ normalized with respect to systemic hematocrit (H₀sys); H₀sys, discharge hematocrit; H₀/H₀sys, H₀ normalized with respect to H₀sys; CTRL, control; PLASMA, group hemodiluted with homologous plasma; HES, group hemodiluted with hydroxyethyl starch solution; DIL, the two hemodiluted groups.
creases along the consecutive complete flow cross sections and is more pronounced in the hemodilution experiments compared with control conditions. The difference is statistically significant (t test, p<0.01) for all cross sections above eight in the experimental data and above 11 in the model results. This indicates that hemodilution causes the development of a stronger positive correlation between hematocrit and flow velocity along the consecutive complete flow cross sections.

Application of the hydrodynamic flow model to the experimental data allows the extension of the analysis to parameters requiring the knowledge of volume flow that was not experimentally available. Figure 4 compares the flow-weighted average H₀ of the blood passing through the capillaries and arterioles of a given generation. In both CTRL and DIL groups, the general pattern is similar: the main arterioles of the proximal generations carry blood with a hematocrit close to the H₀ inflow. These vessels give rise to capillary side branches that exhibit reduced hematocrits caused by phase separation. As a consequence, the hematocrit increases in the continuing arteriolar vessel tree. Because this process continues up to the most distal vessel generations, there is a substantial hematocrit buildup along the arteriolar vessel tree, and the capillaries in the latest generations exhibit H₀ values exceeding the inflow value. This leads to a preferential distribution of red blood cells to capillaries with high generation numbers and therefore to the long flow pathways. This so-called pathway effect²⁹ is much stronger after hemodilution than in control conditions, as indicated by a hematocrit increase by about 68% of the systemic value after hemodilution compared with 35% for the control. Hence, hemodilution enhances longitudinal hematocrit heterogeneity between proximal and distal capillaries in hemodiluted networks.

In spite of the hematocrit increase with generation number, mean capillary erythrocyte flow decreases from the most proximal to the most distal generations in the control situation (Figure 5). This is due to the dominating effect of the decrease in flow velocity along the arteriolar tree.²¹ However, the redistribution of red blood cells to long flow pathways effected by hemodilution will tend to counteract the effect of flow velocity; hemodilution therefore leads to a reduction of the longitudinal heterogeneity of red blood cell flow.

The redistribution of red blood cell flow results from a redistribution of flow velocity and hematocrit to the benefit of distal capillary generations. This is shown in Figure 6, in which a value of unity indicates that the relative change of the respective parameter in the capillaries of a given generation level equals that found in the inflow arteriole of the network on hemodilution. For all parameters, the ratios are close to unity for generations between 9 and 17. This demonstrates that in
the intermediate generations of capillaries the changes of velocity, hematocrit, and red blood cell flow resulting from hemodilution directly reflect the corresponding changes in the main feeding vessels. A comparison between proximal and distal capillaries, however, indicates the longitudinal redistribution of red blood cell and plasma flow. Both flow velocity and H_D show reductions of about 17% in the proximal capillaries during hemodilution, while the more remote capillary segments experience a corresponding increase. As a consequence, the relative erythrocyte flow after hemodilution is elevated (approximately +40%) in the most distal capillaries at the expense (approximately −30%) of the proximal capillaries close to the feeding arteriole.

Discussion

The results presented demonstrate that hemodilution leads to a redistribution of red blood cell and plasma to the various flow pathways through microvascular networks, thereby influencing the transfer characteristics of vascular beds. This in turn affects the residence times for different blood components in the vessels of a network and therefore hematocrits: with hemodilution, average capillary H_T decreases more than H_R, This is accompanied by an increase in overall hematocrit heterogeneity as represented by the coefficient of variation (Table 1). These findings contrast with reports in which an increase of H_T/H_R was found with decreasing H_R. This is at variance with the notion that the increase of H_T/H_R under hemodilution is combined with a “more uniform spatial distribution of RBC throughout the true capillary level.” It is important to investigate the apparent contradiction between these findings and interpretations, since it has been suggested that a more homogeneous distribution of hematocrit is relevant for the clinical effects of hemodilution.

Hematocrit Distribution

The average H_T in the capillaries of a microvascular network may differ from the inflow or the H_R as a result of two major effects.9,30,31 The vessel Fahraeus effect leads to a reduction of individual vessel hematocrit, H_T, relative to the discharge hematocrit, H_D. The network Fahraeus effect, on the other hand, reduces the area-weighted H_D of a complete flow cross section relative to the H_D in the feeding arteriole, H_R.

From in vitro experiments with blood-perfused glass tubes, the vessel Fahraeus effect is known to be more pronounced at reduced hematocrits.32,33 This is explained by the enhanced axial migration of red blood cells.34 If these results are applicable to blood flow in vivo, the vessel Fahraeus effect would tend to decrease H_T/H_R with hemodilution. The expected influence of the vessel Fahraeus effect on H_T/H_R is, however, small. The network Fahraeus effect is generated by a positive covariance of flow velocity and hematocrit in the microvessels constituting a complete flow cross section.36 The correlation between flow velocity and hematocrit, in turn, depends on the extent of phase separation at the diverging arteriolar bifurcations: at a bifurcation, the daughter branch with the higher volume flow gen-

![FIGURE 5. Graph showing red blood cell (RBC) flow (sliding mean±SEM) in capillaries of a given generation divided by the average capillary RBC flow (total RBC flow through the network divided by the number of capillaries in the network).](image1)

![FIGURE 6. Graphs showing flow velocity (top panel), discharge hematocrit (H_D, middle panel), and erythrocyte (RBC) flow (bottom panel) during hemodilution divided by the respective value under control conditions (DIL/CTRL) for capillaries of different generations. The ratios obtained were normalized with respect to the ratio between the respective parameters in the feeding arterioles (sliding mean±SEM). Asterisks indicate significant difference from unity (two-tailed t test, p<0.01).](image2)
erally receives blood with an increased hematocrit and vice versa.\textsuperscript{28,37} This phase separation effect generates a positive covariance between hematocrit and flow velocity in the daughter branches of a bifurcation and also among vessels constituting the consecutive flow cross sections of a network. The reduction of the mean area-weighted $H_n$, normalized with respect to $\bar{H}_m$ by the network Fahraeus effect therefore increases with the number of branch points passed and shows a maximum at the capillary level (Figure 3).

Earlier investigations have demonstrated that phase separation increases with decreasing hematocrit.\textsuperscript{28,38,39} It can therefore be expected that hemodilution will enhance the network Fahraeus effect and increase heterogeneity of hematocrit distribution. The expected changes are quantitatively described by the flow model and are in close agreement with those found in the experimental data. This indicates that the experimental data presented here for complete networks and their dependence on systemic hematocrit can be explained by the basic rheological mechanisms incorporated into the model, i.e., the Fahraeus-Lindqvist effect and phase separation at arteriolar bifurcations.

The obvious discrepancy between the present results and the majority of data available in the literature may in part be related to the fact that in these studies measurements have been made in a limited number of vessels rather than in complete networks. In larger arteriolar vessels, an increase in hematocrit heterogeneity as well as the concomitant decrease in $H_c/H_m$ after hemodilution might not be found, since phase separation is not prominent in vessels of this size.\textsuperscript{11,14} If, on the other hand, the analysis is based on measurements in capillaries,\textsuperscript{15-19} the selection procedure used will have a strong influence on the results.\textsuperscript{20} In most experimental situations, exact morphological or topological information is not available, and therefore the decision whether a vessel segment is considered a capillary bears some degree of uncertainty. Specifically, a nonrandom selection procedure may favor distal capillaries because of their low flow velocities, thereby leading to an underestimation of the prevalent heterogeneity. Such an unbalanced selection could also influence the magnitude and even the direction of changes in the measured parameters with hemodilution. The present measurements, for example, indicate that a determination of $H_c/H_m$ in distal capillaries would suggest an increase with hemodilution despite the fact that the overall capillary $H_0/H_m$ decreases (Table 1, Figure 6).

Although the discrepancies to previous reports cannot be rigorously accounted for, the validity of the general conclusion presented in this study for complete networks is supported by the compatibility between experimental data and modeling results that are based on accepted rheological laws. In addition, similar reactions on hemodilution could be shown for both the topologically defined group of capillaries and for the subgroup of capillaries with diameters less than 8 $\mu$m (Figure 2). In this morphologically defined subgroup, which might be more closely related to the capillaries as defined in other studies, the majority (approximately 85\%) of hematocrit measurements was made by direct cell counting, a method that was also used in most of the studies cited from the literature.

In reconciling the present study with previous reports in the literature, a number of possible sources for discrepancies can be identified. In addition to the strategy used for selecting vessel segments discussed above, the choice of species and tissue, the hemodilution protocol, and the methods used for hematocrit measurement may be relevant. Preparation technique and the hemodilution protocol could also influence changes of vascular tone accompanying the reduction on systemic hematocrit, depending on the species and tissue used. Such changes, which are absent in the present preparation, would influence distribution of red blood cell flow pathways through microvascular networks by redistribution of volume flow. Finally, the intensity of red blood cell aggregation might affect cell plasma separation at microvascular bifurcations and therefore hematocrit distribution. If hemodilution is carried out with a diluent altering red blood cell aggregation, this may introduce additional effects that are not seen in the present experiments with a species that has extremely low red blood cell aggregation tendency.

**Cell Flow Distribution**

In the mesentery, the capillaries of the different generations are distributed in an onion skin–like pattern around an arteriole (Figure 7). Capillaries with high generation numbers are located in distant regions of the tissue relative to the input arteriole and vice versa. On average, the pressure gradient per capillary will therefore decrease with increasing generation level. The resulting low flow rate in the distal capillaries also leads to a low red blood cell flow despite high hematocrits (Figure 5). This distribution of red blood cell flows is modified by hemodilution: capillaries of high generation levels feeding the most distant areas of the tissue get an increased share of the total red blood cell flow through the network (Figure 6). Because these vessels exhibit the lowest absolute values of erythrocyte flow in the control state, the redistribution tends to homogenize the dispersion of erythrocyte flow to the different tissue regions.

The homogenization of red blood cell flow distribution to different tissue regions is, however, not accompanied by a reduction of the overall heterogeneity of red blood cell flow in the capillaries. In contrast, the coefficient of variation of red blood cell flow increases during hemodilution (Table 2). Only the “longitudinal” component of the coefficient of variation ($CV_l$), i.e., the heterogeneity between proximal and distal capillaries of a network, decreases on hemodilution. This longitudinal heterogeneity is caused by the asymmetrical topological structure of the microvascular bed and by the cumulative effect of phase separation at successive bifurcations. One way to quantify longitudinal heterogeneity is to correlate a given parameter determined for all capillaries with their generation numbers and calculate the respective coefficient of correlation. Based on this correlation coefficient, $CV_l$ can be determined from the systematic variance of the parameter X with capillary generation number. As shown in Table 2, the correlation coefficient of the correlation between red blood cell flow and generation shifts to less negative values after hemodilution, leading to a decrease in the absolute value of the respective correlation coefficient and the
longitudinal heterogeneity described by CV. The changes in red blood cell flow distribution are due to a weaker negative correlation between velocity and generation corresponding to a decrease in CV, while in parallel a more positive correlation between hematocrit and generation is established and the corresponding value for CV increases.*

The values of CV are low compared with the overall coefficient of variation, despite the substantial longitudinal dispersion demonstrated in Figure 6. This reflects the uneven frequency distribution of capillary generation levels. As shown in Figure 8, the largest number of capillaries is found in the intermediate generation range (between eight and 16), where longitudinal redistribution of red blood cell flow with hemodilution is minimal. The comparatively low number of capillaries with low or high generation numbers limits the influence of longitudinal hematocrit redistribution on CV. However, changes of red blood cell flow in these subgroups of capillary vessels are functionally relevant, since capillaries of given generation levels almost exclusively supply specific tissue regions (Figure 7).

The discrepancy between the effects of hemodilution on overall and longitudinal heterogeneity of red blood cell flow shows that an exclusive analysis of global statistical parameters might miss functionally relevant phenomena because of the complex relations among morphological, topological, and hemodynamic parameters within a microvascular network.

In tissues in which adjustments of vascular tone can occur in response to changes of supply conditions, the redistribution pattern described here may be modified. However, in a number of pathological states such as

*The absolute values for the coefficient of variation of capillary flow velocity reported here for the rat mesentery are considerably higher than the average values of about 0.6 given in the literature for striated muscle.\(^{19,20,40}\) This might be related to differences in the angioarchitecture of the investigated tissues and to the fact that in the present study strict topological criteria were used to define capillaries.

**TABLE 2.** Heterogeneity of Velocity, Hematocrit, and Red Blood Cell Flow in the Capillaries of Three Networks Analyzed Using the Hydrodynamic Flow Model

<table>
<thead>
<tr>
<th>Group</th>
<th>Velocity (\text{H}_D)</th>
<th>(\text{Q}_{\text{RBC}})</th>
<th>(\text{r}_{(\text{XGEN})})</th>
<th>(\text{H}_D)</th>
<th>(\text{Q}_{\text{RBC}})</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL</td>
<td>1.65±0.74</td>
<td>0.52±0.02</td>
<td>-0.44±0.07</td>
<td>0.08±0.14</td>
<td>-0.25±0.14</td>
<td>0.64±0.36</td>
</tr>
<tr>
<td>DIL</td>
<td>1.62±0.62</td>
<td>0.68±0.07</td>
<td>-0.39±0.09</td>
<td>0.18±0.11</td>
<td>-0.16±0.14</td>
<td>0.52±0.36</td>
</tr>
<tr>
<td>DIL/CTRL</td>
<td>1.01±0.06</td>
<td>1.31±0.10</td>
<td>0.89±0.10</td>
<td>1.98±1.05</td>
<td>0.57±0.22</td>
<td>0.64±0.24</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CV, overall coefficient of variation; \(\text{r}_{(\text{XGEN})}\), correlation coefficient for the correlation between the respective parameter \(X\) and the generation number; CV, longitudinal component of coefficient of variation (to calculate CV, the residual variance of the parameter X with respect to the generation was subtracted from its overall variance; CV, was then given as the square root of that longitudinal variance divided by the mean value of \(X\); HD, discharge hematocrit; Q_{RBC}, red blood cell flow; CTRL, control; DIL, the two hemodiluted groups.

**FIGURE 7.** Diagrams of the arteriolar vessel tree in the rat mesentery. Solid lines indicate arteriolar segments; dashed lines indicate capillaries. Left panel: The generation number of every capillary is given. Right panel: Contour lines have been drawn to indicate tissue regions supplied by capillaries of the generation numbers indicated at the bottom of the panel.
chronic ischemia, the remaining vassodilative reserve is probably minimized or not recruitable. Improvements of tissue oxygenation by hemodilution in the absence of vasomotor adjustments, which have for instance been demonstrated in skeletal muscle with chronically reduced blood supply,41,42 must be attributed to passive rheological mechanisms. These mechanisms are on one hand related to the effect of hematocrit on viscosity and oxygen-carrying capacity of the blood (“optimal hematocrit”) and on the other hand to the effect of hematocrit on the distribution of red blood cell flow in microcirculatory networks as described here. 

References

Figure 8. Graph showing frequency distribution of generation level for capillary segments in the four networks used for model calculations (n=587, mean=12.8, CV=0.334).
Redistribution of red blood cell flow in microcirculatory networks by hemodilution.
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