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Erythrocyte margination and sedimentation in skeletal muscle venules

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Bishop, Jeffrey J., Patricia R. Nance, Aleksander S. Popel, Marcos Intaglietta, and Paul C. Johnson. Erythrocyte margination and sedimentation in skeletal muscle venules. *Am J Physiol Heart Circ Physiol* 281: H951–H958, 2001.—Previous studies in skeletal muscle of the dog and cat have shown that venous vascular resistance changes inversely with blood flow and may be due mainly to red blood cell aggregation, a phenomenon present in these species. To determine whether red blood cell axial migration and sedimentation contribute to this effect, we viewed either vertically or horizontally oriented venules of the rat spinotrapezius muscle with a horizontally oriented microscope during acute arterial pressure reduction. With normal (nonaggregating) rat blood, reduction of arterial pressure did not significantly change the relative diameter of the red blood cell column with respect to the venular wall. After induction of red blood cell aggregation in the rat by infusion of Dextran 500, red blood cell column diameter decreased up to 35% at low pseudoshear rates (below ~5 s^{-1}); the magnitude was independent of venular orientation. In vertically oriented venules, the plasma layer was symmetrical, whereas in horizontally oriented venules, the plasma layer formed near the upper wall. We conclude that, although red blood cell axial migration and sedimentation develop in vivo, they occur only for larger flow reductions than are needed to elicit changes in venous resistance.

venous resistance; low shear flow; in vivo blood rheology; blood sludging; red blood cell core

NUMEROUS IN VITRO STUDIES of human blood under a variety of experimental conditions have shown that red blood cell aggregation is largely responsible for its significant nonlinear rheological properties. Studies in rotational viscometers have reported an increased apparent viscosity at low shear rates (<5 s^{-1}) due to the formation of red blood cell aggregates (10, 12, 13). Reports from experiments in small glass tubes have shown that in vertically oriented tubes, increased aggregation at low (<15 s^{-1}) pseudoshear rates promotes the formation of a cell-free plasma layer near the tube wall, which tends to maintain the apparent blood viscosity nearly independent of flow rate (24). In contrast, aggregate formation at low (<10 s^{-1}) pseudoshear rates in horizontally oriented tubes is associated with a sedimentation of the high-viscosity red blood cell column to the bottom of the tube and a corresponding increase in apparent blood viscosity at low flow rates (25). It is, therefore, apparent that the non-Newtonian properties of blood can lead to different rheological behavior depending on the geometry and orientation of the flow system. However, the measurements of blood viscosity in glass tubes were made in steady-state conditions, and other studies have shown that the characteristic times of axial migration and sedimentation are longer than that of aggregation and may be sufficient to preclude their significant development in the circulation (1–3, 14, 17).

In an earlier study from our laboratory (11), it was demonstrated that red blood cell aggregation is responsible for increasing venous vascular resistance as mean arterial pressure and blood flow rate are reduced. We (8) recently showed that aggregation causes significant blunting of venular velocity profiles at pseudoshear rates lower than 40 s^{-1} with possible effects seen up to 90 s^{-1}. In that report, it was shown that blunting of the velocity profile could increase the apparent viscosity of blood by up to 100% between the shear rates of 90 and 5 s^{-1} if a significant redistribution of the red blood cells due to axial migration had not occurred. On the basis of the above cited in vitro studies, we hypothesized that axial migration and sedimentation would only be present at lower shear rates and would, therefore, not be significantly involved in changing venous vascular resistance over this shear rate range.

The purpose of this study, therefore, was to determine the conditions under which red blood cell sedimentation and a cell-free layer occur in vivo. Because rat red blood cells do not normally aggregate, Dextran 500 was infused into the animal to induce aggregation intermediate between human and that found in certain other species (cat and dog; see Ref. 8). Using a horizontally oriented microscope with a fully rotatable stage, we oriented microcirculatory venules of the rat spino-

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trapezius muscle in either vertical or horizontal orientations and viewed the red blood cell column in these venules during acute arterial pressure reduction with hemorrhage to reduce flow. For comparison, the hemorrhage procedure was also done with normal animals not treated with Dextran 500.

MATERIALS AND METHODS

Experimental setup. The experimental preparation, data acquisition, and data analysis methods for this study are essentially identical to those described previously (7), and we refer the reader to that work for a complete description. Seventeen male Sprague-Dawley rats (Simonson; Gilroy, CA) weighing between 200 and 300 g (255.8 ± 26.9 g) were used for these investigations. Animal handling and care were provided following the procedures outlined in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The study was approved by the local Animal Subjects Committee.

Rats were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital sodium (Abbott) with additional anesthetic administered throughout the experiment as needed. We exteriorized the spinotrapezius muscle of the anesthetized rat while maintaining the region of the blood supply intact. A tracheal tube was inserted to assist breathing, the jugular vein was catheterized for the administration of anesthetic or dextran during the course of the experiment, and the carotid artery was catheterized to withdraw blood as needed for pressure reductions. Catheters were also placed in both the femoral artery and abdominal vena cava (through the femoral vein) for pressure measurements. All catheters were filled with a solution of heparinized saline (30 IU/ml) to prevent clotting.

Because the microscope stage was oriented vertically and is rotatable, a sling made of flexible clear plastic was developed to affix the animal securely to a Plexiglas platform. Spring-loaded clips were used to secure the sling to the animal platform and thumbscrews were used to secure the animal platform to the microscope stage.

A Leitz metallurgical microscope was oriented horizontally and the animal was mounted on a rotating stage with *x*- and *y*-axis drives to allow horizontal and vertical translation of the muscle as well as rotation. This enabled us to select suitable skeletal muscle venules for study and to then align the axis of these vessels vertically or horizontally. The muscle preparation was transilluminated by a 35-W DC light source (Bausch & Lomb) with red, blue, or green filters alone or in combination to achieve optimal contrast for each preparation. The image was projected onto a black-and-white, charged-coupled device video camera (SSC-M370, Sony) connected to a videocassette recorder (SLV-R1000, Sony) and viewed on a monitor (SSM-121, Sony). Leitz UM20 [0.33 numerical aperture (NA)] and UM32 (0.30 NA) objectives were used along with a UM20 (0.33 NA) condenser lens to provide total full screen magnifications of the image of ×850 (310 μm horizontal) and ×1250 (205 μm horizontal) for the ×20 and ×32 objectives, respectively.

Pressure, hematocrit, aggregation, and velocity measurements. The femoral artery and abdominal vena cava catheters were attached to pressure transducers (TNF-R, Viggo Spectramed), and the transducer outputs were connected to a strip-chart recorder (Brush 2600, Gould). Pressure data were transferred to a microcomputer (300 MHz Pentium II; Micron) either directly on-line during the experiment or more commonly entered manually from the strip-chart recordings at a later time. During the experiment, the transducer zero reference was adjusted as needed when the elevation of the muscle changed with animal rotation.

The hematocrit and degree of red blood cell aggregation were measured during the control period as well as after infusion of Dextran 500. Hematocrit was determined with a microhematocrit centrifuge (Readacrit, Clay Adams). The degree of red blood cell aggregation (M) was assessed from duplicate measurements on a 35 μl blood sample with a photometric rheoscope (aggregometer, Myrenne; Roetgen, Germany) on the 10-s setting.

Red blood cell velocity measurements during hemorrhagic hypotension were obtained using the dual-slit technique of Wayland and Johnson (27) modified for analysis of video images as described by Intaglietta et al. (21). This video method has a maximum velocity limit of ~1.5 mm/s, which precluded its use at normal arterial pressures for the venules viewed in this study. The mean velocity was calculated using the equation: mean velocity (V) = dual-slit velocity/1.6 (5). Reduced velocity ($\bar{u}$), or pseudoshear rate, was calculated using the equation $\bar{u} = V/D$, where D is the vessel diameter.

Venular wall and red blood cell column diameter measurements. The average diameter of the red blood cell column was determined off-line during videotape playback using the image-shearing technique of Intaglietta and Tompkins (22). Similar to the procedure described in a previous article (7), changes in venular wall diameter were determined by following identifiable visual landmarks within the vessel wall. Such landmarks, not necessarily located on the innermost surface of the wall and the inner margin of the wall, were often indistinct. In instances when the innermost dimensions of the venular wall could be clearly visualized, the venular inner diameter was determined at the same location as the red blood cell column diameter to evaluate the hypothesis of the existence of a cell-free layer in these vessels. All measurements of venular wall and red blood cell column diameter were performed by one investigator to maintain consistency of measurement. Determination of the existence and/or width of a cell-free layer by comparison of venular wall and red blood cell column diameters were performed separately by three investigators.

As described below in *Experimental protocol*, the reduction of pressure with blood withdrawal occurred during ~90 s. The rate of the subsequent reduction in flow rate varied among animals; therefore, it was not possible to determine a general relationship for the time dependence of changes in red blood cell column diameter. The diameter of the red blood cell column was measured at 15-s intervals throughout the hemorrhage protocol, and in every instance, a steady-state value of red blood cell column diameter was reached within the observation time of ~2 min. All values of red blood cell column diameter during hemorrhagic hypotension reported here were measured after development of steady-state conditions.

Experimental protocol. After the animal was mounted on the microscope stage, an arterial blood sample (35 μl) was taken to determine control values of hematocrit and aggregation index (M). The microcirculation of the spinotrapezius muscle was inspected, and venules in the diameter range of 13–185 μm were selected for study based on the criteria of stable flow as well as clear focus and contrast of the image. The branching and flow patterns in the vicinity of the selected venules were recorded on videotape for later analysis. Venules were chosen so that the control diameters (range and mean) within the experimental groups comprising the normal and dextran-treated venules were not significantly ($P > 0.05$) different from one another. Similarly, control diameters of horizontally and vertically oriented venules

were not significantly ($P > 0.05$) different from one another. A video image of the vessel was recorded under control conditions for ~2 min, and blood was then removed from the animal via the carotid artery into a heparinized syringe until the mean arterial pressure was ~40 mmHg. An average of 4.4 ± 1.4 ml of blood was withdrawn at a rate of ~3.0 ml/min, after which the reduced pressure was maintained for ~2 min. The blood was then reinfused into the animal over a period of ~1 min. The diameter, pressure, and velocity (until out of range) were monitored until the animal regained a steady-state blood pressure, at which time a new vessel was selected and the protocol repeated. In nine experiments, the animal was euthanized with an infusion of 300 mg/kg pentobarbital sodium before the reinfusion procedure, and red blood cell column dimensions were monitored until a steady-state value was reached after cessation of flow.

The hemorrhage protocol described above was repeated for each venule selected with either normal (nonaggregating) blood or after infusion of Dextran 500 (200 mg/kg body wt) to induce red blood cell aggregation. The dextran (average molecular mass 460 kDa; Sigma) was dissolved in saline (6%) and infused in 50 mg/kg increments during 2–3 min. On the basis of a total blood volume of 5.5% (1), an average hematocrit of 40%, and an average body weight of 254 g, this represents a plasma dextran concentration of ~0.6%. Hematocrit and aggregation index (M) values were determined 15 min after dextran infusion. In only one rat was a discernable adverse reaction to the dextran infusion manifested by swelling of the limbs; but no significant differences in blood pressure, blood flow, or vessel response were seen.

Statistical analysis. There were four sample groups to be compared as follows: horizontally oriented normal vessels; horizontally oriented vessels with dextran infusion; vertically oriented normal vessels; and vertically oriented vessels with dextran infusion. Vessels were chosen to provide each of the four sample groups with a similar distribution within the vessel diameter range studied.

All data are reported as means ± SD. The statistical significance of changes in red blood cell column diameter were measured by comparing the absolute values of control measurements to those taken during hemorrhagic hypotension or after blood reinfusion using both the paired *t*-test and the nonparametric Wilcoxon signed-rank test. Sample groups were compared against each other for differences in control pressures and diameter means and ranges using both the *t*-test and the nonparametric Mann-Whitney rank sum test. An ANOVA test was used to test for differences between distributions of red blood cell column diameter changes, and the coefficient of variation (SD/mean) was calculated for changes in each group. An ANOVA test with a subsequent Bonferroni *t*-test was used to test for differences in red blood cell column diameter change for vessels of different sizes. Statistical tests were done using a commercially available software package (SigmaStat, Jandel Scientific). Regression curves represent the best-fit relationship to the experimental data as determined by an automated curve-fitting software program (TableCurve 2D, Jandel Scientific). Comparison of regression lines to determine significance was performed using the standard procedures outlined by Glantz (18). For all tests, $P < 0.05$ was considered statistically significant.

RESULTS

Hematocrit and aggregation. The hematocrit of normal rats was 41.9 ± 4.2%, and the index of aggregation was 0.0 in all cases. In dextran-treated rats, the hematocrit was 39.5 ± 3.7%, and the index of aggregation was 9.6 ± 4.0. The mean hematocrit of the dextran-treated rats was not significantly ($P > 0.05$) different from that of normal animals.

Arterial and venous pressures with hemorrhage. Mean arterial pressures in the control state were 112.0 ± 24.5 and 117.8 ± 27.8 mmHg for normal and dextran-treated rats, respectively. Corresponding mean venous pressures were 5.8 ± 1.8 and 7.3 ± 2.3 mmHg for normal and dextran-treated rats, respectively. During hemorrhagic hypotension, mean arterial pressures were 41.1 ± 9.9 and 38.7 ± 14.2 mmHg and mean venous pressures were 4.8 ± 1.4 and 4.8 ± 1.6 mmHg for normal and dextran-treated rats, respectively. On reinfusion of shed blood, mean arterial pressures were 123.3 ± 23.0 and 130.1 ± 27.9 mmHg and mean venous pressures were 6.7 ± 1.6 and 7.3 ± 2.6 mmHg for normal and dextran-treated rats, respectively.

Red blood cell velocities. At control arterial pressures, red blood cell velocities in nearly all venules studied were out of the range of the velocity system (~1.5 mm/s). On reduction of arterial pressure to 40 mmHg, mean red blood cell velocities in the venules of normal animals averaged 0.23 ± 0.23 mm/s, which was not significantly ($P > 0.05$) different from the average of 0.20 ± 0.26 mm/s for dextran-treated animals. For the vessels studied, this is equivalent to pseudoshear rates of 3.7 ± 3.6 and 2.7 ± 2.6 s^{-1} for the normal venules and dextran-treated animals, respectively. Mean cellular velocity at an arterial pressure of 40 mmHg was significantly ($P < 0.001$) correlated to venular diameter for both normal and dextran-treated animals (Fig. 1*A*), but there was no correlation ($P > 0.05$) between the corresponding pseudoshear rate and vessel diameter for either normal or dextran-treated animals (Fig. 1*B*).

Cessation of blood flow occurred in only 3 of 131 venules studied (1 normal, 2 dextran treated). The mean arterial pressure in these cases was not significantly ($P > 0.05$) different from the group as a whole.

Absence of a cell-free layer at control arterial pressure. The inner boundary of the venular wall was often indistinct; therefore, an exact comparison of vessel inner diameter and red blood cell column diameter was not always possible. Given this limitation, our observations did not reveal a cell-free plasma layer near the venular wall at control arterial pressure. Red blood cell movement could be observed immediately adjacent to the vessel wall for both normal and dextran-treated animals. In a sample group of venules where the inside margin of the venular wall was evident, the inner diameter of the venular wall was determined and compared with the diameter of the red blood cell column. For these venules, the difference between the venular diameter and the column diameter was 0.8 ± 0.4 μm for normal vessels ($n = 7$) and 0.9 ± 0.4 μm for dextran-treated vessels ($n = 7$). These values are not significantly different from one another ($P > 0.05$) and are less than the resolution of our microscope system (~1 μm).

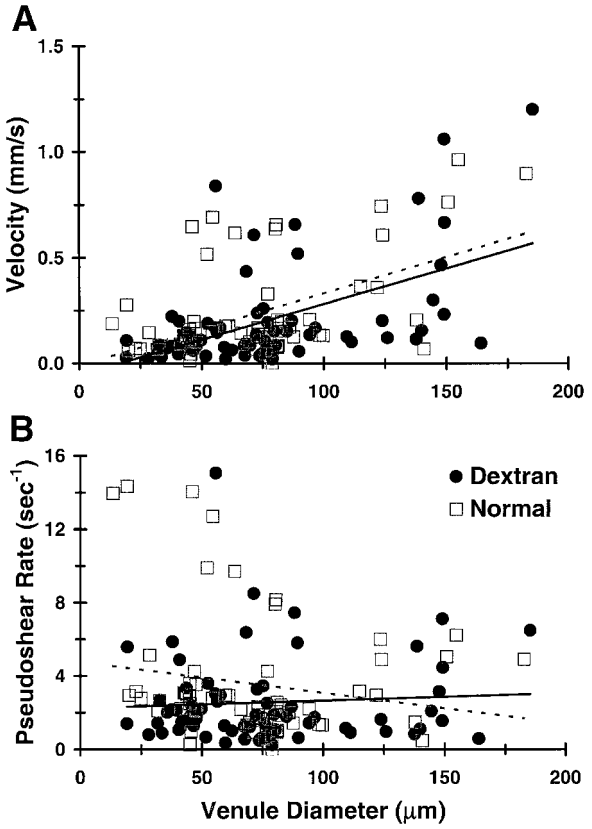

Fig. 1. Mean red blood cell (RBC) velocities (*A*) and corresponding pseudoshear rates (*B*) at mean arterial pressure (P_A) = 40 mmHg. Velocity was significantly ($P < 0.001$) correlated to venular diameter for both normal and dextran-treated animals. There was no correlation ($P > 0.05$) between pseudoshear rate and vessel diameter for either normal (dashed lines) or dextran-treated animals (solid lines).

Changes in venular diameter with arterial pressure reduction. Changes in diameter of the red blood cell column during hemorrhagic hypotension were normalized relative to the control column diameter. Therefore, to properly understand the hemodynamic effect of such changes it is important to also consider the corresponding changes in venular wall diameter. A reduction in diameter of −0.9 μm (1.3% of vessel diameter) was seen in venules of normal animals, a value not significantly different ($P > 0.05$) from −1.9 μm (2.8% of vessel diameter) for venules of dextran-treated animals. Diameter reduction in venules oriented vertically (−1.8 μm; 2.4% of vessel diameter) was not significantly different ($P > 0.05$) from venules oriented horizontally (−1.0 μm; 1.7% of vessel diameter).

Changes in red blood cell column diameter with arterial pressure reduction. In normal animals, there was no net movement of red blood cells away from the venular wall during reduction of arterial pressure and blood flow with hemorrhage. In contrast, a retraction of the red blood cell column was seen in a number of the dextran-treated venules on reduction of arterial pressure. When the venule under study was oriented horizontally, this retraction manifested itself as sedimentation. When the venule under study was oriented vertically, the retraction of the red blood cell column resulted in a cell-free layer on both sides of the column, which was normally axisymmetrical.

Relationship between red blood cell column diameter and shear rate. The diameter of the red blood cell column relative to the control value is shown as a function of pseudoshear rate for horizontally (Fig. 2*A*) and vertically (Fig. 2*B*) oriented venules. As expected, the slope of the regression line for normal animals is not significantly ($P > 0.05$) different from zero for venules of either orientation, indicating no shear rate dependence in the absence of red blood cell aggregation.

For dextran-treated blood, it can be seen in Fig. 2, *A* and *B*, that the large (>10%) retractions in red blood cell column diameter occur primarily at the lowest shear rates investigated. As expected, there is a significant dependence of red blood cell column retraction on shear rate for dextran-treated blood for venules oriented both horizontally (Fig. 2*A*) and vertically (Fig. 2*B*). This retraction does not become significantly ($P > 0.05$) larger than the retraction of the venular wall until a pseudoshear rate <5 s^{-1} is obtained. Again, it is

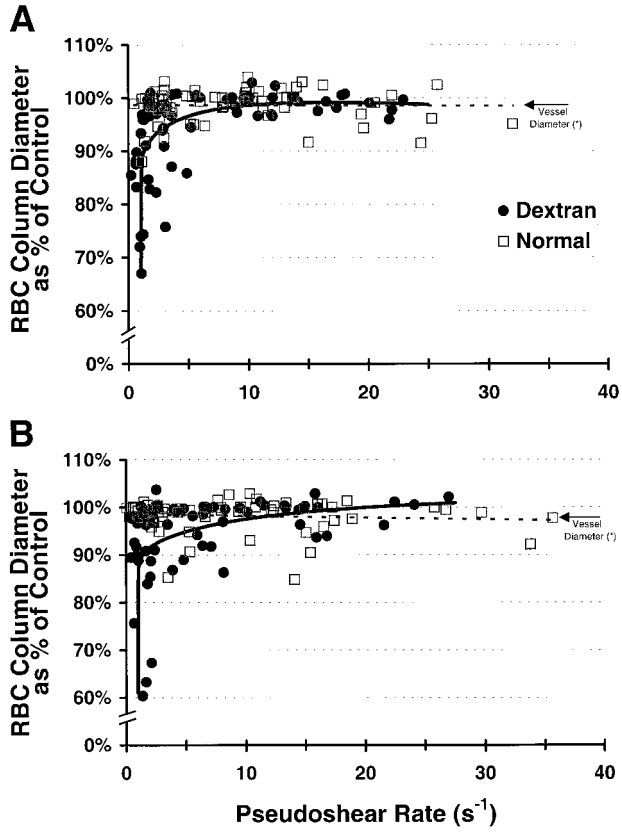

Fig. 2. Normalized RBC column diameters for normal and dextran-treated blood in venules oriented horizontally (*A*) and vertically (*B*) vs. pseudoshear rate. Regression lines for both normal (dashed lines) and dextran-treated blood (solid lines) are not significantly ($P > 0.05$) different with venular orientation. The slopes of the regression lines for normal blood are not significantly ($P > 0.05$) different from zero, indicating no dependence on shear rate in the absence of RBC aggregation. Regression lines for dextran-treated blood become significantly different than for normal blood at pseudoshear rates <5 s^{-1} independent of venular orientation.

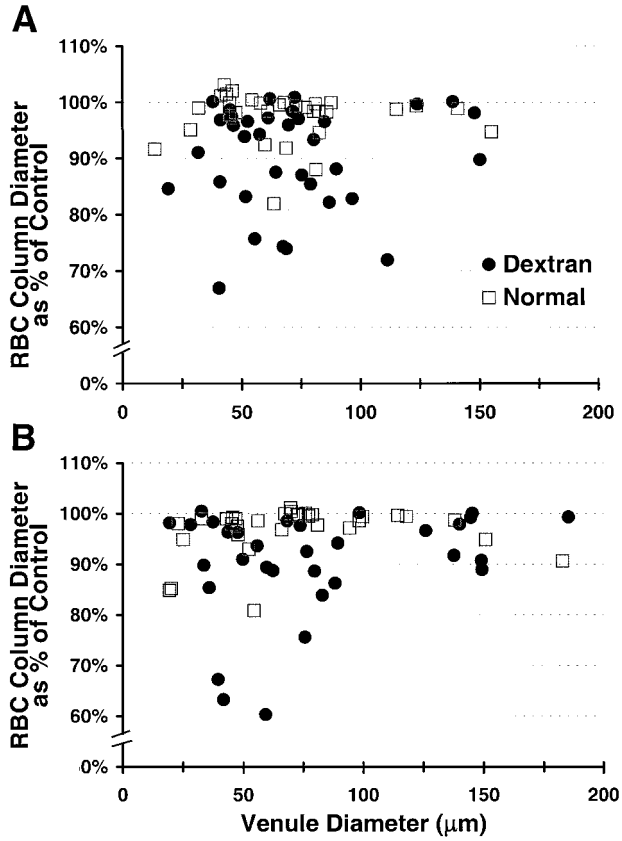

Fig. 3. Normalized RBC column diameters at P_A = 40 mmHg vs. venular diameter at control pressure for normal and dextran-treated blood in venules oriented horizontally (*A*) and vertically (*B*). Diameter decreases in dextran-treated blood are significantly ($P < 0.001$) larger than for normal blood as also seen in Fig. 2. There is no correlation between the decrease in RBC core diameter and venular diameter in either dextran-treated or normal blood.

interesting to note that the regression lines for both normal and dextran-treated blood are not significantly ($P > 0.05$) different for venules oriented horizontally compared with venules oriented vertically, meaning that the shear rate dependence of red blood cell column retraction is independent of venular orientation.

Relationship between red blood cell column diameter and venular diameter. The pooled data showing the effect of hemorrhagic hypotension are shown for horizontally (Fig. 3*A*) and vertically (Fig. 3*B*) oriented venules. For normal blood, the reduction in column diameter in horizontally oriented venules (-1.7 ± 2.9 μm; 2.5% of vessel diameter) was not significantly different ($P > 0.05$) from that in vertically oriented venules (-1.9 ± 3.5 μm; 3.2% of vessel diameter). This retraction of the red blood cell column was not significantly different ($P > 0.05$) from the reduction in the wall diameter in these venules. This is consistent with our visual observation that development of a cell-free layer did not occur with normal blood during the experimental protocol. In the few cases where a larger decrease in diameter did occur, it was due to flow disruption or stasis in an upstream side branch, which caused a disturbed flow pattern rather than the steady-state development of a cell-free marginal layer. The slope of the regression lines for normal blood is not significantly ($P > 0.05$) different from zero for the venules of either orientation, signifying no correlation between the magnitude of column diameter reduction and venular diameter.

Also shown in Fig. 3 is the effect of hemorrhagic hypotension on red blood cell column diameter with dextran-treated blood. The average decrease in column diameter in horizontally oriented venules (-6.0 ± 6.9 μm; 9.7% of vessel diameter) was not significantly different ($P > 0.05$) from that in vertically oriented venules (-6.0 ± 5.9 μm; 9.4% of vessel diameter). The magnitude of these reductions in column diameter are significantly ($P < 0.001$) larger than the reductions in venular wall diameter. The slopes of the regression lines for dextran-treated blood in Fig. 3, *A* and *B*, are not significantly different (>0.05) from zero, indicating no relationship between venular diameter and the magnitude of red blood cell column reduction with hemorrhagic hypotension.

A summary of the changes in red blood cell column diameter with hemorrhagic hypotension is shown in Table 1. It is interesting to note that the magnitude of retraction in the diameter of the red blood cell column was independent of venular orientation for both dextran-treated and normal animals. This fact suggests that the phenomena of erythrocyte axial migration and sedimentation are caused by similar hemodynamic forces, the only difference being the location of the red blood cell column relative to the vessel wall, which is influenced by the presence of gravitational forces in the horizontally oriented venules.

Red blood cell column diameters with reinfusion. On reinfusion of shed blood, the diameter of the red blood cell column increased in diameter in each vessel studied. This increase was nearly identical in magnitude to the retraction in diameter seen during hemorrhagic hypotension, so that there was no significant ($P = 0.91$) difference between the control and postreinfusion diameters for the venules of any group. The time course of this phenomenon was rapid, reaching steady state within 15 s of completion of blood reinfusion.

Table 1. *Summary of changes in steady-state red blood cell column diameter with hemorrhagic hypotension*

Experimental Group	*n*	Δ*D*, μm	Δ*D*, %	% Smaller	Pseudoshear Rate, s^{-1}
Normal	64	-1.8 ± 3.2	-2.9 ± 4.6	86	3.7 ± 3.6
Dextran	67	-6.1 ± 6.4	-9.5 ± 9.8	90	2.7 ± 2.6
Normal-Horz	32	-1.7 ± 2.9	-2.5 ± 4.4	84	4.1 ± 3.7
Normal-Vert	32	-1.9 ± 3.5	-3.2 ± 4.9	91	3.4 ± 3.5
Dextran-Horz	35	-6.2 ± 6.9	-9.7 ± 9.3	89	2.3 ± 1.8
Dextran-Vert	32	-6.0 ± 5.9	-9.4 ± 10.5	91	3.0 ± 3.2
Euthanized	9	-9.8 ± 4.8	-11.6 ± 7.1	100	0.7 ± 0.5

Values are means ± SD; *n*, number of rats. Δ*D*, red blood cell column diameter change; %smaller, the percentage of venules in each group where the red blood cell column diameter decreased with hemorrhagic hypotension. Horz, horizontally oriented vessels; vert, vertically oriented vessels. All venules in the euthanized group were dextran treated.

DISCUSSION

Principal findings. At control arterial pressure, red blood cells appeared to be immediately adjacent to the venular wall within the resolution of the optical system (~1 μm). This was true for both normal (nonaggregating) blood and dextran-treated blood. On reduction of arterial pressure to 40 mmHg, the red blood cell column of normal (nonaggregating) blood showed only a small (−2.9%) reduction in diameter. This change was essentially identical to the reduction in venular diameter and was independent of orientation. This finding, coupled with visual observations, suggests that even during severe reductions in arterial pressure, a cell-free layer did not develop in the absence of red blood cell aggregation.

For dextran-treated blood, the red blood cell column diameter decreased significantly more than did the venular wall at pseudoshear rates <5 s^{-1}. It is noteworthy that the relationship between the column diameter change and the pseudoshear rate is independent of venular orientation as shown in Fig. 2 and Table 1. When a reduction in column diameter occurred in horizontally oriented venules, red blood cells settled to the bottom wall of the venule and a cell-free plasma layer formed above the red blood cell column. The width of this layer averaged ~7% of vessel diameter (~4.2 μm) at pseudoshear rates of 5 s^{-1} and below but rose to nearly 35% of the vessel diameter at the lowest pseudoshear rates (~1 s^{-1}). In vertically oriented venules, this same magnitude of reduction produced a symmetrical cell-free plasma layer near both walls of the venule. At a pseudoshear rate of 5 s^{-1}, this plasma layer averaged ~4% of vessel diameter (~2.1 μm) and rose to 20% in the most extreme instance at the lowest pseudoshear rates (~1 s^{-1}).

Limitations of measurement. As discussed previously (6), the limit of horizontal and vertical video resolution associated with this experimental setup is ~1 μm. The optical resolution based on the wavelength of light and the numerical aperture of the lenses is ~0.7–0.8 μm. The image-shearing system used was shown by Intaglietta and Tompkins (22) to have an accuracy for repeated measurements of 0.5% of the total image width. On the basis of these factors, we estimate the uncertainty associated with our measurements to be ~1 μm.

Red blood cell column diameter changes in nonaggregating blood. With normal (nonaggregating) blood, the average retraction of the red blood cell column was −1.8 μm (−2.9%) as arterial pressure was reduced to 40 mmHg compared with a venular diameter change during arterial pressure reduction to 40 mmHg of −1.4 μm (−2.0%) (7). The difference between these two changes is not significantly different ($P > 0.05$) from zero and is less than the limit of resolution of our system, leading to the conclusion that the changes in red blood cell column diameter for normal blood are due to narrowing of the venule and not to a movement of the red blood cells away from the venular wall. These findings are in agreement with those of Cokelet and Goldsmith (15), who reported no net inward migration of nonaggregating human red blood cells flowing through glass tubes over a similar range of pseudoshear rates (0.15–50 s^{-1}).

Red blood cell column diameter changes in aggregating blood. For dextran-treated blood, the present results show an average retraction of the red blood cell column of −6.1 μm (−9.5%) during arterial pressure reduction to 40 mmHg. This change, which is independent of orientation, is significantly ($P < 0.001$) larger than the reduction in venular wall diameter of −1.9 μm (2.8%) seen in these vessels (7). This net movement of the red blood cell column away from the venular wall produces a cell-free plasma layer, which is completely above the sedimented red blood cell column in horizontally oriented venules and split into apparently symmetrical plasma layers on either side of the red blood cell column in vertically oriented venules.

Using normal human blood (aggregation index, M = 16), Reinke and colleagues (24, 25) noted that retraction of the red blood cell column in 29–94 μm vertically oriented glass tubes became significant at pseudoshear rates <5–15 s^{-1} and normalized column diameters were 83% and 72% of control at pseudoshear rates of 5 and 1 s^{-1}, respectively, compared with values of 95% and 88% in our study. Studies with hyperaggregating blood show that both the shear rates where significant retraction begins and the magnitude of the column retraction at specific pseudoshear rates are larger (15, 24, 25), indicating that column retraction is directly related to the aggregation tendency of the blood. Because the dextran-treated rat blood used in this study has an aggregation tendency somewhat less than that for normal human blood (M = 9.6 vs. 16), our results are consistent with expectation.

In a companion study, we show that the rate of axial migration of cells in aggregating blood increased as shear rate was reduced, but we did not observe the formation of a visible cell-free layer near the walls of venules in this preparation, even on reduction of shear rate to 6–8 s^{-1} (9). We observed that the magnitude of radial dispersions caused by intercellular collisions decreases with decreasing shear rate (6). Although axial migration forces tend to push cells toward the central axis of the vessel, dispersion of cells within the red blood cell core pushes cells radially outward in opposition to the axial migration forces. Dispersion force dominates at control arterial pressure, but it appears that the threshold value where axial migration forces become dominate and contraction of the red blood cell core begins occurs at a pseuoshear rate of ~5 s^{-1}. As reported in RESULTS, the average systemic hematocrit was 40%. On the basis of the correlation between systemic hematocrit and the local hematocrit in venules of this size given by Lipowsky et al. (23), it is estimated that local hematocrit in the venules of the present study was ~ 25%. At reduced hematocrits, sedimentation would occur at higher shear rates than those observed here.

Time dependence of red blood cell aggregation. A number of in vitro studies have investigated the time

dependence of aggregation, axial migration, and sedimentation using a variety of experimental methods. Cokelet (14) reported that the first visible signs of aggregation in human blood do not occur until 5–12 s after a step reduction in shear rate to a low (<7 s^{-1}) value and that development of steady state takes up to 2 min. Although the rate is roughly dependent on tube or vessel diameter, the time required for significant development of either sedimentation or a cell-free marginal layer is between 20 and 60 s (1–3). The effect of the difference in time dependence between aggregation and axial migration or sedimentation was investigated by Alonso et al. (3) and Gaehtgens (17), who calculated apparent blood viscosity in horizontally and vertically oriented glass tubes (26–83 μm diameter) after 10 s and again after 300 s. Apparent blood viscosity increased in both tubes regardless of orientation after 10 s but decreased in the vertically oriented tubes after 300 s, suggesting that the characteristic time for axial migration was longer than 10 s.

Because both the critical shear rate at which significant retraction of the red blood cell column occurs and the kinetics of column retraction are correlated to the aggregation tendency of blood, it would be expected that both the dextran-treated rat blood of the present study and normal cat blood, both of which show less aggregation tendency than human blood, would have characteristic times for axial migration and sedimentation that are at least as long, if not longer than, those reported for human blood. Engelson et al. (16) gave a quantitative description of the anatomic arrangement of the collecting venules in this muscle and showed that the total path length between the smallest postcapillary venules and the large venular arcade was ~1,800 μm. By combining these data with the relationship between venular length and diameter obtained by us in the companion study (9), we found that the transit times, from capillaries to arcade venules at control arterial pressure where the pseudoshear rate is 100 s^{-1} or more, are <1 s. If 5 s^{-1} is taken as a threshold pseudoshear rate value, the resulting transit time of ~10 s is in close agreement with the characteristic times of aggregation obtained in the previous in vitro studies (3, 14, 17). This result supports our hypothesis that in the control situation there is insufficient residence time in the venular network for either axial migration or sedimentation to develop to a significant degree. These phenomena would have little effect on venous vascular resistance until the pseudoshear rate is reduced to a low value.

Implications for venous vascular resistance. Previous studies have shown that skeletal muscle venous vascular resistance increases by >100% on arterial pressure reduction to 40 mmHg (11, 26). However, the small change in venular diameter seen in this preparation would increase resistance by only 8% (7). The increase in resistance was almost zero in the absence of red blood cell aggregation (11). We recently showed that blunting of velocity profiles in venules due to red blood cell aggregation is significant at 40 s^{-1} and may begin at pseudoshear rates <90 s^{-1}. The effects of this blunting could increase venous resistance by up to 100% over the same pressure range if the apparent blood viscosity near the wall was not significantly altered by the formation of a cell-free layer at the wall with aggregation (8). Sedimentation of the red blood cell column in horizontally oriented venules would increase effective blood viscosity and could also affect resistance. However, the present study demonstrates that significant axial migration and sedimentation occurred only at pseudoshear rates <5 s^{-1}. In species such as dogs or cats, where the aggregation tendency is less than for the dextran-treated rat observed in this study, retraction of the red blood cell core away from the venular wall may begin at slightly lower pseudoshear rates. In vitro studies suggest that this would not be a large change (15, 24, 25), but it would further diminish the effects of axial migration and sedimentation in the pseudoshear rate range at which changes in resistance have been observed. Although most venules in the body are not normally oriented in any preferential direction, the finding of the present study shows that the magnitude of the retraction of the red blood cell column with shear reduction is independent of orientation. On the basis of this result, it may be concluded that such behavior is relevant to venules of any orientation, and that axial migration and sedimentation are not important determinants of venous vascular resistance over the physiological shear-rate range where changes in venous resistance have been shown to take place. However, because these phenomena were observed at pseudoshear rates <5 s^{-1}, they are likely to have an effect under pathological conditions (i.e., ischemia-reperfusion, sepsis, etc.) where blood flow is greatly reduced.

Another factor suggested as a possible cause of the observed changes in venous vascular resistance with flow is a change in the number of vessels with flow. Cessation of blood flow in our study occurred in only 2.3% of the venules studied. House and Johnson (19, 20) reported the occurrence of flow stoppage in <10% of the venules when mean arterial pressure was reduced to 20 mmHg. Similar to those findings, we observed that cessation of blood flow was not due to constriction of the venules in the area of stasis. Because stasis occurred so infrequently and was not apparently related to the presence of red blood cell aggregation, this factor probably does not play a significant role in the increased venous vascular resistance seen as arterial pressure is reduced.

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