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Diameter changes in skeletal muscle venules during arterial pressure reduction

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Bishop, Jeffrey J., Patricia R. Nance, Aleksander S. Popel, Marcos Intaglietta, and Paul C. Johnson. Diameter changes in skeletal muscle venules during arterial pressure reduction. *Am J Physiol Heart Circ Physiol* 279: H47–H57, 2000.—Previous studies in skeletal muscle have shown a substantial (>100%) increase in venous vascular resistance with arterial pressure reduction to 40 mmHg, but a microcirculatory study showed no significant venular diameter changes in the horizontal direction during this procedure. To examine the possibility of venular collapse in the vertical direction, a microscope was placed horizontally to view a vertically mounted rat spinotrapezius muscle preparation. We monitored the diameters of venules (mean diameter 73.8 ± 37.0 μm, range 13–185 μm) oriented horizontally and vertically with a video system during acute arterial pressure reduction by hemorrhage. Our analysis showed small but significant ($P < 0.0001$) diameter reductions of 1.0 ± 2.5 μm and 1.8 ± 3.1 μm in horizontally and vertically oriented venules, respectively, upon reduction of arterial pressure from 115.0 ± 26.3 to 39.8 ± 12.3 mmHg. The venular responses were not different after red blood cell aggregation was induced by Dextran 500 infusion. We conclude that diameter changes in venules over this range of arterial pressure reduction are isotropic and would likely increase venous resistance by <10%.

venous resistance; venous pressure; venous collapse; venous wall architecture; hemorrhage

THE TWO PRIMARY FUNCTIONS of the venous network are to provide postcapillary resistance and the major capacitance of the vascular bed. Many studies have shown that capillary pressure remains relatively constant during reduction of arterial pressure (14, 18, 22, 27, 42), and evidence indicates that this stabilization of capillary pressure is due to blood flow autoregulation and changes in venous resistance (8, 18, 21). Studies on skeletal muscle (6, 22, 36) and isolated mesentery (13, 14) showed that the rise in venous vascular resistance occurs outside the range of blood flow autoregulation with resistance increasing as flow falls below normal levels and decreasing during hyperemia.

The factors that may cause changes in venous resistance can be inferred from Poiseuille's equation for viscous flow in a tube, $R = \Delta P/Q = 8l\eta/\pi r^4$, where ΔP, Q, l, r, and η are the pressure difference between upstream and downstream points, the fluid flow rate, tube length, tube radius, and the viscosity of the fluid, respectively. In an earlier study of cat skeletal muscle, we showed that blood viscosity changes due to red blood cell aggregation are responsible, at least in part, for changes in venous resistance with flow (6). However, because vessel radius is raised to the fourth power in the Poiseuille relationship, it is clear that even small changes in diameter could have an important effect on resistance. A previous study done in this laboratory (17) showed no significant diameter changes in cat skeletal muscle venules during arterial pressure reduction to 40 or 20 mmHg. That study was done on a horizontally mounted preparation, so the possibility of venular collapse in the vertical direction still remained.

The purpose of this study, therefore, was to obtain information on the question of collapse of the microcirculatory venules by monitoring the vertical diameter of venules aligned in the horizontal plane during arterial pressure reduction by blood withdrawal. For comparison, data were also obtained on venules aligned vertically. This study was done using a muscle preparation in the rat, an animal whose blood normally shows negligible red blood cell aggregation tendency (4, 5). Because red blood cell aggregation may alter pressure distribution in the venous network, the study was repeated after addition of Dextran 500 (referred to hereafter as Dextran). Additionally, in the context of capacitance, these data on venular diameter changes in skeletal muscle during hemorrhage provide information on the contribution of this vascular bed to the overall blood loss.

MATERIALS AND METHODS

Microscope setup. A Leitz metallurgical microscope was oriented horizontally as shown in the lower portion of Fig. 1. The animal was mounted on a rotating stage with X-Y drives to allow horizontal and vertical translation of the muscle as well as rotation. This enabled us to select suitable skeletal

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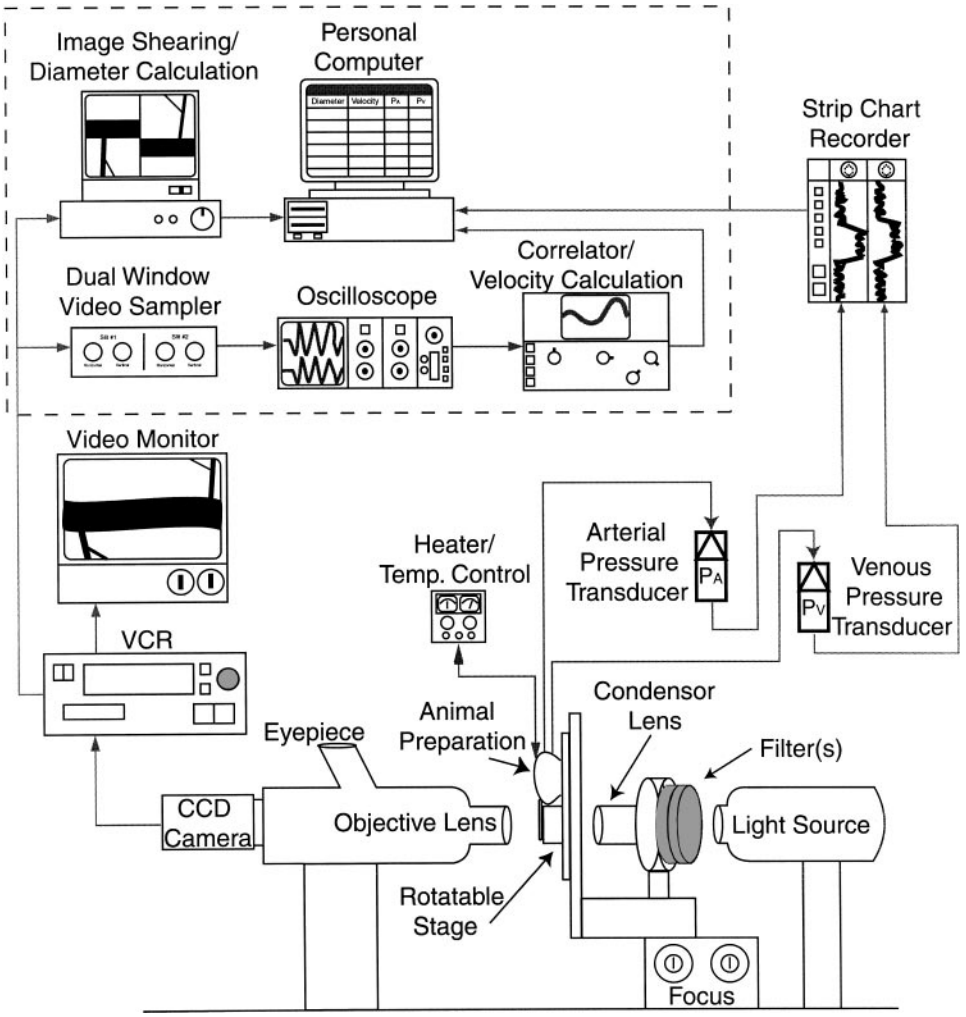

Fig. 1. Schematic diagram of the horizontal microscope experimental setup. Dashed line denotes measurements (red blood cell velocity and venular diameter) taken offline from videotape playback. CCD, charge-coupled device.

muscle venules for study and to then align the longitudinal axis of these vessels either horizontally or vertically. The muscle preparation was transilluminated by a 35-W DC light source (Bausch & Lomb). Red, blue, or green filters were used alone or in combination to provide optimal viewing contrast for each preparation. The optical image was projected onto a black and white CCD video camera (SSC-M370, Sony) connected to a videocassette recorder (SLV-R1000, Sony) and viewed on a monitor (SSM-121, Sony). Leitz UM20 (numerical aperture 0.33) and UM32 (0.30) objectives were used along with a UM20 (0.33) condenser lens, a setup that provided for total full-screen magnifications of the image of $\times$850 (310 μm horizontal) and $\times$1,250 (205 μm horizontal) for the $\times$20 and $\times$32 objectives, respectively.

Surgery. Twenty-four male Sprague-Dawley rats (Simonson, Gilroy, CA) weighing between 200 and 300 (254.0 $\pm$ 27.1) g were used for these investigations. Animal handling and care were provided following the procedures outlined in the *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). Additional anesthetic was administered throughout the experiment as needed. The animal was placed on a heating pad to maintain body temperature during surgery. A tracheal tube was inserted to assist breathing, the jugular vein was catheterized for 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. In addition, catheters were 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.

An exteriorized rat spinotrapezius muscle preparation similar to that described previously (32) was used for these studies. The skin was opened to expose the spinotrapezius muscle. A drip of Plasma-Lyte A, adjusted to pH 7.4 (Baxter), was maintained throughout surgery to keep the muscle moist. Connective tissue was cleared from the surface of the muscle, and the muscle was separated from surrounding tissue with the region of the blood supply intact. Size 4-0 sutures were attached to the outer edges of the muscle to be later affixed to the microscope stage.

Because the microscope stage was oriented vertically and is rotatable, a sling was developed to affix the animal securely to a Plexiglas platform. The sling was made of flexible clear plastic to allow for monitoring the respiration and anesthesia state of the animal throughout the experiment. Spring-loaded clips were used to secure the sling to the animal platform. The muscle was then secured onto the platform using the attached sutures and periphery wax (Surgident, Miles). Moist gauze was placed along the edges of the muscle and petroleum jelly applied to the gauze. Plasma-

Lyte A was suffused over the muscle which was then covered with a polyvinyl film (Saran Wrap, Dow Corning) while air bubbles were removed from its surface. A temperature probe was placed beside the muscle, and temperature was maintained at 37°C by regulation of a heating element attached to the animal platform. Thumbscrews were used to secure the animal platform to the microscope stage.

Pressure 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 fed into a microcomputer (300 MHz Pentium II, Micron) either directly online during the experiment or more commonly inputted manually from the strip-chart recordings at a later time. During the course of the experiment, the transducer zero reference was adjusted as needed when the elevation of the muscle changed with the rotation of the animal.

Hematocrit and aggregation measurements. The hematocrit and degree of red blood cell aggregation were measured during control as well as after infusion of Dextran 500. Hematocrit was determined after centrifugation with a microhematocrit centrifuge (Readacrit, Clay Adams). The degree of red blood cell aggregation was assessed from duplicate measurements on a 0.35-μl blood sample with a photometric rheoscope (Myrenne Aggregometer, Myrenne, Roetgen, Germany). The use of this technique as well as comparisons of this index of aggregation (M) with other methods and between animal species have been described previously by Baskurt et al. (4, 5). The aggregation index on the 10-s setting was used for these investigations.

Diameter measurements. Diameters were measured offline from videotape playback using the image shearing technique of Intaglietta and Thompkins (19). Because of slight irregularities of the inner vessel wall, each measurement of diameter for a particular vessel was performed at the same axial location on the vessel throughout the entire arterial pressure change protocol. Visual landmarks on the vessel wall were followed throughout the course of viewing on a particular vessel so that changes in diameter could be more accurately noted. All diameter measurements were performed by one investigator to maintain consistency of measurement.

Velocity measurements. Red blood cell velocity measurements during hemorrhagic hypotension were obtained using the dual-slit technique of Wayland and Johnson (40) modified for analysis of video images as described by Intaglietta et al. (20). 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 (V) was calculated using the equation: V = dual-slit velocity/1.6 (3). Reduced velocity (U), or pseudoshear rate, was calculated using the equation $U = V/D$, where D is the vessel diameter.

Experimental protocol. Before the investigations were started, control values of hematocrit and aggregation index were determined from a 0.35-μl blood sample. Skeletal muscle 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. 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 to determine the effect of further pressure reduction. In eight experiments, arteriolar and venular diameters in a horizontally oriented muscle were monitored with a vertically oriented microscope during topical administration of 3.0 ml papaverine HCl (10^{-3} M; Sigma) through a small catheter placed under the polyvinyl film during preparation of the muscle.

The hemorrhage protocol described above was repeated after infusion of Dextran 500 (200 mg/kg body wt) to induce red blood cell aggregation. The Dextran (average mol mass 460 kDa, Sigma) was dissolved in saline (6%) and infused in 50 mg/kg increments over the course of 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 values were determined 15 min after Dextran infusion. In only one rat a discernable adverse reaction to the Dextran infusion was manifested by swelling of the limbs, but no significant differences in blood pressure, blood flow, or vessel response resulted.

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 an adequate coverage of the vessel diameter range.

All data are reported as means ± SD. The statistical significance of changes in arterial or venous pressure or venular diameter were measured by comparing the absolute values of control measurements to those taken during hemorrhagic hypotension or following 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 vessel 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 pressure at different body axis orientations as well as for differences in diameter change for vessels of different sizes. The chi-square test was used to test for differences from the expected number of vessels getting smaller during hemorrhagic hypotension when vessels were grouped by orientation, initial diameter, or Dextran treatment. Statistical tests were done using a commercially available software package (SigmaStat, Jandel). For all tests, $P < 0.05$ was considered statistically significant.

RESULTS

Arterial and venous pressures with body orientation. Mean arterial pressure and mean venous pressure were monitored for changes due to orientation of the animal body. A reference state with the body axis oriented horizontally was defined as 0°, and measurements were taken at 0°, 90°, 135°, 180°, 225°, and 270° with respect to horizontal (90° = head down; 270° = head up). There were no significant differences (P =

0.324) in mean arterial pressure at any orientation, but there was a significant ($P = 0.005$) decrease in control mean venous pressure at the head-down (90°) position (4.88 ± 2.06 mmHg) compared with both the horizontal (0°) (6.98 ± 1.95 mmHg) and head-up (270°) (7.49 ± 1.67 mmHg) positions. Mean venous pressure during hemorrhagic hypotension was also significantly ($P = 0.002$) less at the head-down position (3.42 ± 1.22 mmHg) than at the horizontal (5.95 ± 1.27 mmHg) or head-up (5.92 ± 1.30 mmHg) positions. Although the mean venous pressure in the head-down position was significantly less than in the horizontal position, body position was unrelated to the orientation of the venule under study.

Hematocrit and aggregation. The hematocrit of normal rats was 42.1 ± 4.2% and the index of aggregation (M) was 0 in all cases. In Dextran-treated rats, the hematocrit was 38.9 ± 2.7%, and the index of aggregation (M) was 9.25 ± 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 Dextran infusion. During infusion of Dextran into the animals, arterial pressure dropped ~20 mmHg, followed by a gradual return over the course of ~5 min to a new steady-state value (117.8 ± 27.8 mmHg compared with 112.0 ± 24.5 mmHg before infusion). This difference was not significant ($P > 0.05$). Venous pressure showed a similar pattern with an immediate decline of about 1 mmHg followed by a rise to a new level that was significantly higher ($P = 0.002$) by 1.45 ± 0.73 mmHg. After post-reinfusion stabilization, there was no difference ($P > 0.05$) in either arterial or venous pressure between normal and Dextran-treated rats.

Arterial and venous pressures with hemorrhage. Figure 2 shows the mean arterial and venous pressures during the control, hemorrhagic hypotension, and reinfusion states. The average reduction in mean arterial pressure was 71.0 ± 27.4 mmHg in normal rats (112.0 ± 24.5 to 41.1 ± 9.9 mmHg) and 79.2 ± 27.1 mmHg in Dextran-treated rats (117.8 ± 27.8 to 38.7 ± 14.1 mmHg). The corresponding reductions in mean venous pressure were 1.1 ± 0.7 mmHg (5.84 ± 1.76 to 4.79 ± 1.44 mmHg) and 2.5 ± 1.4 mmHg (7.29 ± 2.27 to 4.83 ± 1.64 mmHg) for normal and Dextran-treated animals, respectively. Upon reinfusion of shed blood, the mean arterial pressure rose to a steady-state value 10.9 ± 14.8 mmHg higher ($P < 0.0001$) than the control pressure for normal animals and 11.3 ± 13.3 higher ($P < 0.0001$) for Dextran-treated animals. Post-reinfusion venous pressure was 0.9 ± 0.6 mmHg higher ($P < 0.0001$) than control for normal animals, but these values were not significantly different in Dextran-treated animals. There was no difference ($P > 0.05$) in either mean arterial or venous pressure of rats when vessels were oriented horizontally versus when vessels were oriented vertically. This finding was expected because there is no correlation between the vessel orientation and the orientation of the rat body on the stage.

Red blood cell velocities. Mean red blood cell velocities in venules during hemorrhagic hypotension averaged 0.23 ± 0.25 mm/s. For a mean venular diameter of 73.8 μm, this corresponds to a pseudoshear rate (mean velocity/diameter) of 3.1 s^{-1}. Cessation of blood flow occurred in a small number of vessels (6%) but only when mean arterial pressure dropped below 30 mmHg.

Venular diameters with hemorrhage. Figure 3 presents video images from a typical experiment showing a paired venule (*left*) and arteriole (*right*) at control (Fig. 3*A*), during hemorrhagic hypotension (Fig. 3*B*), and after reinfusion of blood (Fig. 3*C*). As demonstrated in Fig. 3, even though the venular diameter is large enough (134.9 μm) that its wall presumably con-

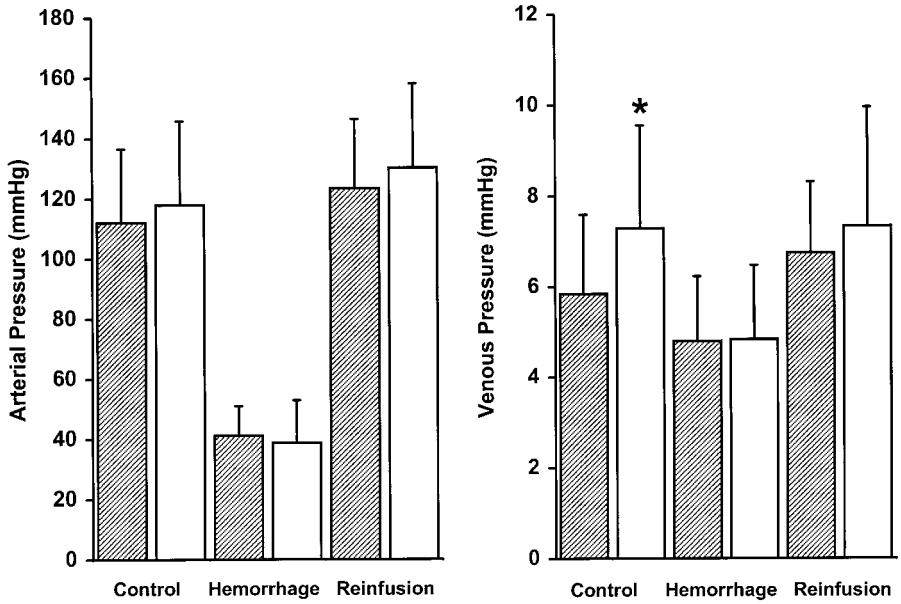

Fig. 2. Mean arterial and vena cava pressures at control, hemorrhage, and reinfusion states (hatched bars, normal; open bars, Dextran). *Significant ($P > 0.05$) compared with normal. Error bars = SD.

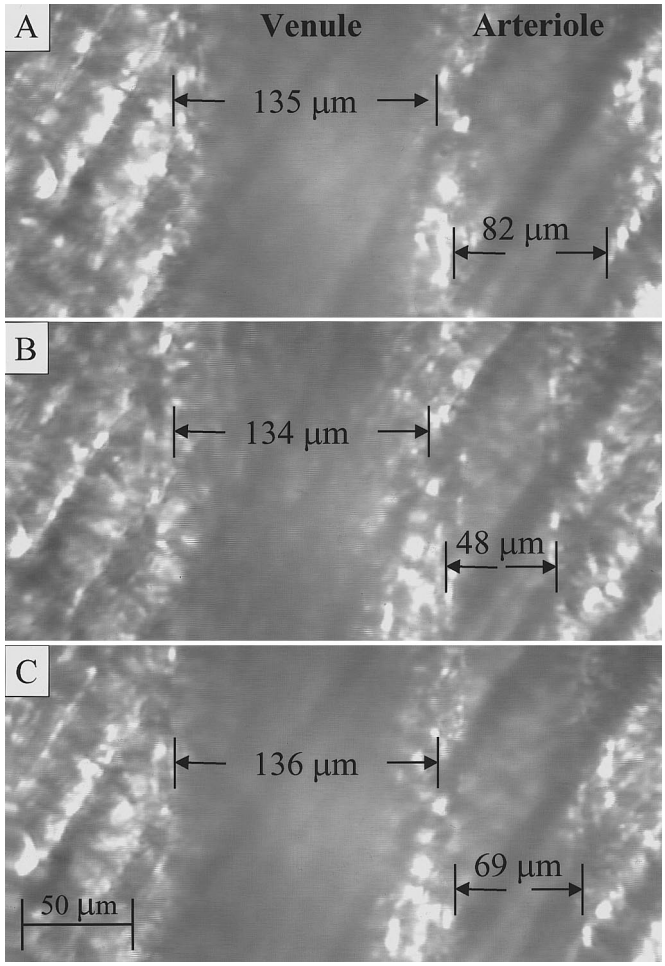

Fig. 3. Video images showing a venule (*left*) and arteriole (*right*) during control (*A*), hemorrhagic hypotension (*B*), and reinfusion states (*C*).

tains smooth muscle, the venular diameter reduced by <1% (to 133.7 μm) during hemorrhagic hypotension, whereas the arteriolar diameter decreased by 42% (82.3 to 47.8 μm).

The pooled results from all investigations are plotted in Fig. 4, which shows the vessel diameter during hemorrhagic hypotension relative to the control diameter in horizontally oriented vessels. Overall, there was a very small but significant ($P < 0.0001$) reduction in vessel diameter (1.0 ± 2.5 μm) during reduction of mean arterial pressure in venules of both normal rats (0.6 ± 2.3 μm) and Dextran-treated rats (1.3 ± 2.5 μm). There was no statistical difference between normal and Dextran-treated rats in either the control diameters ($P = 0.53$) or the magnitude of the diameter reduction during hypotension ($P = 0.19$). Additionally, the slopes of the regression lines for both normal and Dextran-treated rats were not significantly different from zero ($P > 0.05$), indicating no correlation between vessel size and the magnitude of the diameter change seen during hemorrhagic hypotension.

Figure 5 shows the vessel diameter during hemorrhagic hypotension relative to the control diameter in vertically oriented venules. Similar to the horizontally oriented venules, there was a significant ($P < 0.0001$) reduction in vessel diameter of 1.8 ± 3.1 μm upon reduction of arterial pressure by hemorrhage. Diameter reductions in normal (1.2 ± 3.0 μm) and Dextran-treated (2.3 ± 3.1 μm) rats were not statistically different ($P = 0.18$) and neither were the control diameters ($P = 0.51$). As with the horizontally oriented venules, a linear regression analysis confirmed that the slopes for both normal and Dextran-treated rats were not significantly different from zero ($P > 0.05$), indicating no correlation between vessel size and the magnitude of the diameter reduction taking place during hypotension.

The absolute and relative diameter changes observed in this study are summarized by control diameter in Table 1. In each of the groups a small decrease in diameter occurred upon hemorrhage. Only the largest vessels (>140 μm) decreased in diameter to a significantly ($P = 0.0015$) greater degree (4.6 ± 4.5 μm) than the complete data set. This decrease was not

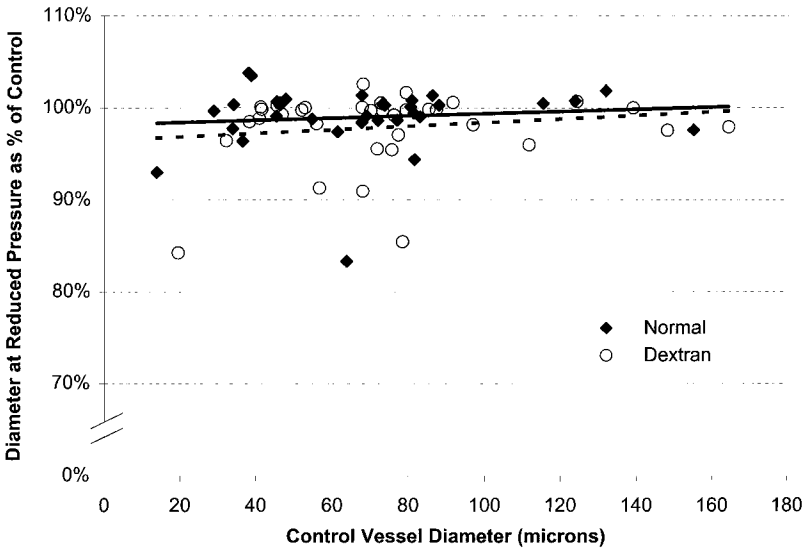

Fig. 4. Relative venular diameters at hemorrhagic hypotension compared with control diameter for horizontally oriented venules of normal and Dextran-treated rats. Slopes of the regression lines for normal (solid line) and Dextran-treated (dashed line) rats are not significantly different from zero ($P > 0.05$).

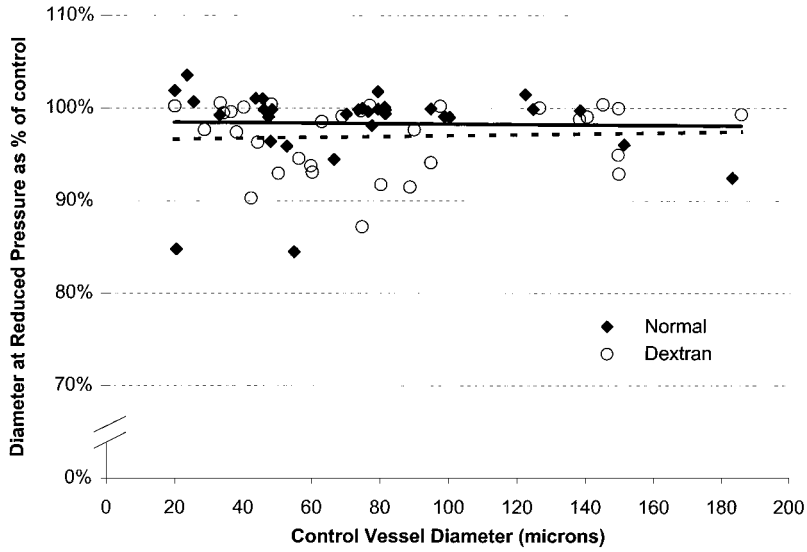

Fig. 5. Relative venular diameters at hemorrhagic hypotension compared with control diameter for vertically oriented venules of normal and Dextran-treated rats. Slopes of the regression lines for normal (solid line) and Dextran-treated (dashed line) rats are not significantly different from zero ($P > 0.05$).

significant ($P = 0.08$), however, when expressed as a percentage of vessel diameter.

Table 1 also shows a summary of the diameter changes for sample groups based on vessel orientation and the presence of Dextran 500. There was no difference ($P = 0.207$) in the magnitude of the diameter change between any of the sample groups. It is noteworthy that the same magnitude of diameter reduction was seen regardless of venule orientation. The diameter reduction seen in vertically oriented venules (1.8 ± 3.1 μm) is slightly larger than the diameter reduction seen in horizontally oriented venules (1.0 ± 2.5 μm), although not significantly so ($P = 0.361$). Additionally, the presence of Dextran in the blood had no significant effect ($P = 0.374$) on the diameter change during pressure reduction.

Whereas the magnitude of diameter changes was small, it was consistent. The last column of Table 1 (% smaller) shows that in virtually all diameter groups, a majority of vessels became smaller during hemorrhagic hypotension and overall 66% of the vessels showed this behavior. A chi-square test showed that this proportion does not vary significantly among vessels grouped by initial diameter ($P = 0.358$), vessel orientation ($P = 0.192$), or Dextran treatment ($P = 0.264$).

Table 1. *Summary of venule diameter changes with hemorrhage*

Control Diameter, μm	n	ΔD, μm	ΔD, %	% Smaller
15–30	9	−0.71	−3.8	67
30–40	13	−0.18	−0.5	69
40–50	20	−0.53	−1.2	60
50–60	10	−2.79	−5.0	90
60–70	13	−1.77	−2.7	77
70–80	23	−1.63	−2.1	74
80–90	14	−1.01	−1.2	64
90–100	7	−1.23	−1.3	71
110–140	11	0.02	0.02	36
140–185	11	−4.56	−2.9	82
Normal	64	−0.91	−1.3	62
Dextran	67	−1.90	−2.8	73
Horizontal	67	−1.03	−1.7	61
Vertical	64	−1.76	−2.4	73
Euthanized	9	−1.17	−0.7	56
All vessels	131	−1.37	−2.0	66

ΔD, diameter change. %Smaller refers to the percentage of venules in each group that decreased in diameter during hemorrhagic hypotension.

A complete overview of the magnitude of individual vessel changes is shown in Figs. 6 and 7. Figure 6 is a histogram of diameter changes for all venules oriented horizontally and vertically. The distribution is negatively skewed for venules of both orientations, resulting in the median values of the diameter changes (−0.5% and −0.6% for horizontally and vertically oriented vessels, respectively) being less negative than the mean values. These median values further illustrate that the diameter reduction occurring during hemorrhagic hypotension is small and independent of vessel orientation. The coefficients of variation (SD/mean) for horizontal and vertical vessels are 2.3 and 1.7, respectively, indicating that the differences in response among individual vessels were greater than the overall trend.

Figure 7 shows the distribution of this change for vessels in normal and Dextran-treated rats. Again, the distribution is negatively skewed (median values −0.2% and −0.9% for normal and Dextran-treated rats, respectively). The coefficients of variation for normal and Dextran-treated rats are 3.0 and 1.4, respectively. The four distributions shown in Figs. 6 and 7 are not significantly different from one another ($P = 0.174$).

In nine rats, euthanasia with pentobarbital sodium (300 mg/kg) was performed after hemorrhage. In these animals, both the arterial and venous pressures equilibrated to a mean pressure of 6.51 ± 0.59 mmHg within 5 min. The nine vessels viewed during this procedure ranged in diameter from 52 to 164 μm. As shown in Table 1, even in this extreme situation the diameter fell only 1.2 ± 2.7 μm (0.7 ± 1.9%). This

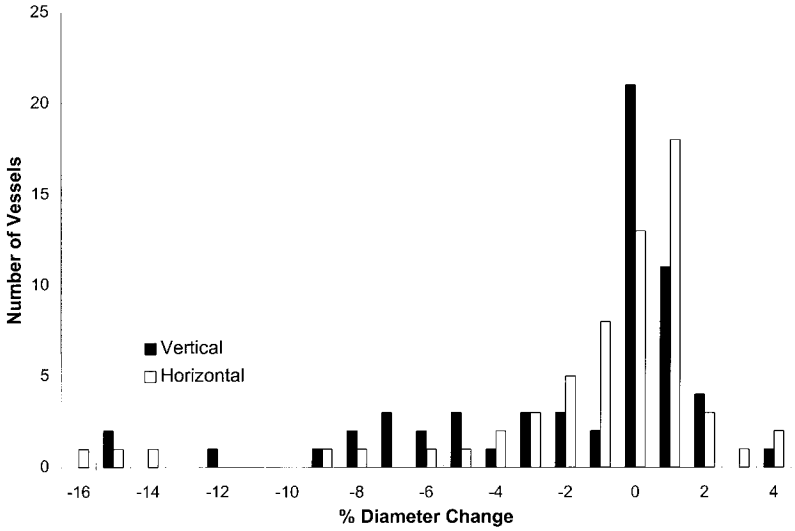

Fig. 6. Histogram distribution of relative diameter changes during hemorrhagic hypotension for horizontally oriented venules of normal and Dextran-treated rats.

reduction was similar in magnitude to that seen with hemorrhage but was not significantly different from control ($P = 0.238$) perhaps because of the small sample size.

Venular diameters with reinfusion. On reinfusion of shed blood, the diameter increased in each group. This increase was nearly identical to the decrease seen during hemorrhagic hypotension for each group, so that there was no significant difference ($P = 0.745$) between the control and post-reinfusion diameters for the venules of any of the groups. The distribution of these changes was also not statistically different ($P = 0.813$) for any of the sample groups.

Active arteriolar and venular diameter changes. In eight experiments, we viewed paired arterioles and venules in a horizontally oriented muscle preparation during a procedure of hemorrhage followed by topical administration of papaverine HCl. In these experiments, arteriolar diameter (control diameter range 45–97 μm) decreased by 12.9 ± 3.7% during hemorrhagic hypotension, whereas venular diameter (control diameter range 49–151 μm) decreased by only 0.31 ± 0.15%. Upon suffusion of the muscle with papaverine HCl, the arteriolar diameter increased by 13.4 ± 5.0%, whereas the venular diameter increased only 0.67 ± 0.49%. Neither change in venular diameter was statistically significant ($P > 0.05$).

DISCUSSION

Principal finding. The salient finding of this study is that the vertical diameter of venules in rat spinotrapezius muscle oriented horizontally decreases very little (1.7 ± 4.0%) with arterial pressure reduction to 40 mmHg (Fig. 4). This same magnitude of response (2.4 ± 4.1%) is seen in venules oriented vertically (Fig. 5), indicating that gravitational effects on venular diameter are not significant over this arterial pressure range. The diameter change was not influenced significantly by the presence of red blood cell aggregation. This diameter reduction would increase vascular resistance by ~8% based on Poiseuille's equation and thus would make only a small contribution to the change in

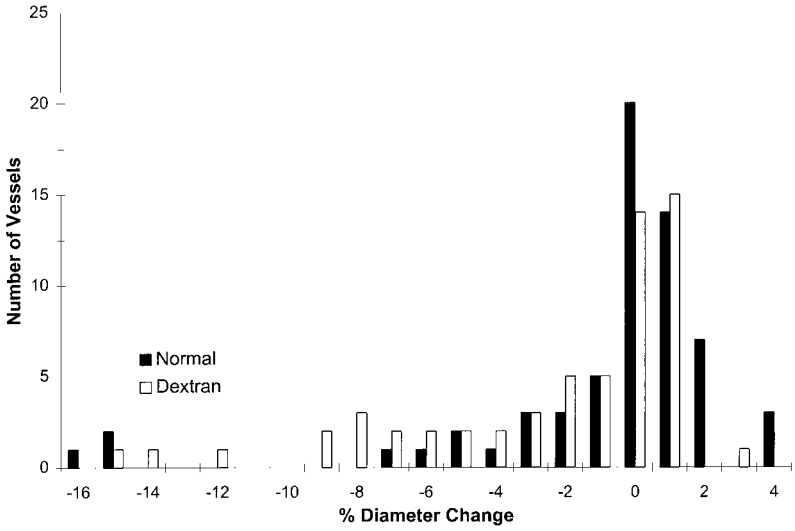

Fig. 7. Histogram distribution of relative diameter changes during hemorrhagic hypotension for vertically oriented venules of normal and Dextran-treated rats.

venous resistance (>100%) previously reported with arterial pressure reduction (6, 36).

Limitations of measurement. The observed change is close to the limit of resolution of our microscope system. The optical resolution, based on the wavelength of light and the numerical aperture of the lenses, is ~0.7–0.8 μm. The vertical video resolution is ~1.0 μm, whereas the horizontal video resolution is also ~1.0 μm due to decreased resolution during video recording (31). The image-shearing system used was shown by Intaglietta and Tompkins (19) 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. The magnitude of the observed changes in vessel diameters (2%) is on the same order or more than the accuracy, therefore the data of diameter changes may reach significance, although they are on the order of the accuracy. As shown in Table 1, roughly two-thirds of the venules decreased in diameter regardless of grouping by initial diameter, venular orientation, or Dextran treatment. This consistent trend supports the observation that a significant decrease in diameter is occurring even though the magnitude of the reduction is small. Visual landmarks on the wall were followed throughout the hemorrhage period so that errors in diameter changes (ΔD) would not be subject to lumen irregularities along the vessel length but rather be dependent on the limits of video and optical resolution.

Estimated venular pressure change. In a previous study of cat sartorius muscle in our laboratory (18), at an arterial pressure of 115 mmHg the venular pressure ranged from 10.2 mmHg in first-order (178.7 ± 41.4 μm) venules to 20.5 mmHg in fourth-order (24.2 ± 7.2 μm) venules. It is reasonable to suppose that venular pressures are similar in our preparation at this arterial pressure.

Other studies (14, 18, 42) show that as arterial pressure changes, the corresponding pressures in the capillaries, venules, and veins do not change in the same proportion. As shown in Fig. 2, in our study, as arterial pressure fell from 115 to 40 mmHg (−65%), the pressure in the vena cava fell 24% (6.3 to 4.81 mmHg). In cat sartorius muscle (18), the change in venular pressure on reduction of arterial pressure from 115 to 40 mmHg by occlusion of the supply artery varied from 32 to 39%. It is likely, however, that the pressure changes in the venules of the present study were considerably greater due to differences in the method of pressure reduction. Using fluorescently labeled red blood cells (39), we have obtained velocities of 6.83 ± 3.85 mm/s (n = 14) under control conditions in venules (diameter range 40–70 μm) in this muscle (unpublished data), whereas as reported above, mean red blood cell velocity during hemorrhagic hypotension was 0.23 ± 0.25 mm/s, a reduction of >96%. Presumably venular pressures would decrease by a similarly large amount during this flow reduction, because in normal animals there is no red blood cell aggregation and hence little change in blood viscosity with flow variation. By contrast, flow fell only by half in the cat sartorius muscle with supply artery occlusion. At such low flow, the pressure in the venular network in our preparation would likely be considerably lower than in the cat sartorius and, at most, several millimeters of mercury above vena cava pressure (approximate range of 5–7.5 mmHg).

In the euthanized animals, complete flow stoppage likely had little additional effect on venular pressure. In these animals, the vena cava pressure of 6.51 ± 0.59 mmHg at flow stasis was probably representative of that throughout the venular network. It is noteworthy that even at these pressures, diameter changes were very small.

Pressure-diameter relations of venous vessels. Öberg (27) found that the diameter of a segment of inferior vena cava did not change as transmural pressure was reduced from 30 to 9 mmHg. Further pressure reduction to 5 mmHg, however, increased horizontal diameter slightly, which Öberg suggested was due to vessel collapse beginning at transmural pressures of 6–8 mmHg. If the mechanical properties of the venules and the vena cava are similar, vertical collapse of the venules with little or no horizontal diameter change would be expected based on Öberg's data. In the euthanized animals, as noted above, venular pressures should approach the vena cava pressure (6.51 ± 0.59 mmHg). It is noteworthy that even in this extreme case, collapse of the venule was not observed, suggesting a significant difference in mechanical properties of skeletal muscle venules compared with the vena cava.

Baez et al. (2) showed that upon elevation of the venous pressure from 9.2 to 27.5 mmHg, a 6% increase in diameter occurred in collecting venules (18–68 μm initial diameter) of the rat mesoappendix. Further elevation of venous pressure to 30 mmHg produced no further distention, suggesting the limit of passive distension had been reached. In Baez's study, venular diameters increased only 4% upon elevation of venous pressure from 10 to 20 mmHg. If a similar decrease in diameter were observed upon return of venous pressure to 10 mmHg, a 40-μm venule would decrease ~1.5 μm in diameter. Given the reduction in venous pressure (6.5–13 mmHg) that likely occurred in the venules of this diameter range in our study, Baez's data predict venular diameter reductions of 3–5%, which is slightly more than the 2% actually observed.

Shoukas and co-workers determined pressure-diameter relationships for rat small intestinal muscle venules (24–97 μm initial diameter) during changes in venous pressure due to hemorrhage or carotid artery occlusion (16, 33). Venular diameters decreased between 3.6 and 13.6% over the venular pressure range expected in our studies and were similar in magnitude regardless of the method of pressure reduction. No significant trend in the magnitude of diameter reduction versus venular diameter was noted in these studies, a feature also noticed in the present study. Even greater changes were reported by Gaehtgens and Uekermann (12) in mesenteric venules (22–148 μm) of the dog; venular diameters increased over 30% when arte-

rial pressure was increased from 0 to 150 mmHg, with venous pressure at zero. The change was linear throughout this pressure range, thus predicting a diameter reduction of ~14% if the arterial pressure was reduced from 115 to 40 mmHg as in our experiments. Overall these studies show diameter changes that are several times greater than those found in our study.

Structure of the venous vessel wall. The differences in effect of pressure changes on venular diameter in skeletal muscle and intestine suggest that the structural elements of the venular wall or surrounding tissue may differ. The structural elements of the venular wall are described in excellent detail in several review articles (28–30, 34, 41). Capillary walls contain only endothelial cells surrounded by a basal lamina (28), whereas the walls of the largest veins contain multiple layers of cells (including smooth muscle) and basal laminae. Between these extremes, venules show a gradual increase in complexity downstream from the capillary network and among the venules of different tissues and species. Generally, venules smaller than 30 μm contain no smooth muscle cells, with these cells gradually appearing in venules up to diameters of about 50 μm. A continuous layer of smooth muscle is found in small veins greater than 300 μm. In venules, smooth muscle is typically arranged in a spiral arrangement distinct from the circular arrangement found in arterioles of similar size (28). There is little or no sympathetic innervation of these venules (34).

Connective tissue elements, particularly collagen, are considerably more abundant in the walls of veins than in the walls of arteries (29). Rothe (30) noted that up to 80% of the vessel wall in small venules is made up of collagen fibrils, whereas in corresponding arterioles the wall is over 50% smooth muscle and less than 20% collagen. Starting with the smallest postcapillary venules, a collar of pericytes and collagenous fibrils between 2 and 3 μm thick surrounds venules. This is true of both the smaller venules without smooth muscle as well as the muscular larger veins. Given the relatively high stiffness of collagen (Young's modulus $\sim 1 \times 10^{10}$ dyn/cm^2) compared with the other connective tissue (e.g., elastin, Young's modulus $\sim 6 \times 10^6$ dyn/cm^2) and cellular (e.g., smooth muscle, Young's modulus $\sim 10^4$ dyn/cm^2) components of the wall (10), it is possible that such an architecture helps to maintain the vessel shape and prevents the collapse of these small venules. In support of this idea, Rothe presents data showing that veins are stiffer (circumferential elastic modulus, $E_\theta \sim 47 \times 10^6$ dyn/cm^2) than arteries ($E_\theta \sim 8 \times 10^6$ dyn/cm^2) at the same pressure (18 mmHg). Because venules are typically larger in diameter than arterioles of corresponding orders yet have thinner walls, this combination creates a larger circumferential tension in the venular wall (Law of Laplace) and reduces the ability of smooth muscle to contract against distending pressures.

An additional factor that may explain the relative rigidity of the vessel wall to transmural pressure changes was proposed by Fung et al. (11), who suggested that capillaries behave as tunnels in a gel and that their mechanical stiffness is derived mainly from the properties of the surrounding tissue in which they are located. If we consider a venule to be a pressurized cylindrical tunnel in an infinite elastic medium, we obtain the solution (37)

$$\frac{R}{R_o} - 1 = (1 + \nu) \cdot \frac{P}{E}$$

where R_o is the unstressed radius, R is the pressurized radius, P is the internal pressure, and E and ν are the Young's modulus and Poisson's ratio, respectively, of the surrounding media. Because $\nu = 0.5$ (incompressible medium) and $E \approx 6 \times 10^6$ dyn/cm^2 for relaxed muscle (11), pressure values, P, of 3 and 13 mmHg give $R/R_o - 1 = 0.1\%$ and 0.4%, respectively. This solution demonstrates that the surrounding tissue may contribute appreciable stiffness to the vessel. The extension of this hypothesis to venules is supported by Rothe (30), who postulated based on his earlier findings that veins embedded in tissue may be sufficiently tethered by surrounding tissue elements that collapse does not occur even with large negative transmural pressures. Considering the findings of Fronek and Zweifach (8), who found that pressures in the mesenteric collecting venules (25–50 μm) of the cat are higher than those in venules of the same diameter range found in skeletal muscle (20.7 ± 1.5 to 11.5 ± 0.6 mmHg), it is possible that the correspondingly higher pressures in the mesentery help maintain the distended shape of these venules despite having less structural support from the surrounding tissue.

Active responses of venules. Previous studies have reported a myogenic response to pressure elevation and flow-induced dilation in venules containing smooth muscle (7, 23–25). Dörnyei et al. (7) demonstrated a weak myogenic response in large rat gracilis muscle isolated venules (340 μm), which moderately reduced the large diameter increases (87%) induced by changes in transmural pressure from 0.5 to 17.5 mmHg. Koller et al. (23) demonstrated a flow-induced 20% increase in diameter of 179-μm venules preconstricted with norepinephrine. Kuo et al. (24) found even larger increases (24%) in isolated porcine subepicardial coronary venules (80–120 μm) with flow.

In our studies, the myogenic response and flow-induced dilation could act in opposition to arterial pressure reduction and tend to minimize diameter changes. However, this seems unlikely because in the situations where a vasodilator (papaverine HCl, 10^{-3} M) was topically suffused over the muscle, arteriolar diameter increased to a significant degree, whereas no significant change was observed in venular diameter. In addition, venular diameter was monitored throughout the hemorrhage protocol of all experiments, and no transient biphasic diameter changes were observed, as might be expected if two separate active mechanisms were engaged. These observations combined with the fact that the percent diameter changes were similar in the largest venules, which presumably contain smooth muscle, and the smallest, which would not, suggest

that active mechanisms do not operate to a significant degree in the venules of this muscle.

Effects of Dextran and aggregation. In our studies, infusion of Dextran 500 (200 mg/kg) induced a significant degree of aggregation [the M value as measured with the Myrenne aggregometer rose to 9.25 ± 4.01 compared with 5.07 ± 1.25 normally present in the cat (6)], but arterial pressure was not significantly higher either before hemorrhage or after reinfusion. Vena cava pressure rose significantly with Dextran infusion, but the effect was not seen after reinfusion. By contrast, Mchedlishvili et al. (26) reported that Dextran 500 increased the mean arterial pressure by more than one-third, but the amount of Dextran infused on a body weight basis was five to eight times higher than the amount infused in our studies. Whatever pressure change may have occurred in the venules with aggregation in our studies, it did not significantly affect the response of the vessel to arterial pressure reduction.

Effect of body position. In our studies, the rat body axis was rotated to orient a venule either horizontally or vertically. Stepke et al. (35) reported a rise in the mean arterial pressure of rats with long-term orientation in the head-down position, but this change became statistically significant only after 14 days. In our studies this position was never maintained for more than 30 min, and no significant change in arterial pressure was observed. It is possible that certain body orientations could influence venous pressure in the region draining the muscle. Our data show differences of <1 mmHg in pressure at the level of the muscle as a function of body position, which would have little effect on diameter of the venules. More importantly, there was no relation between body position and vessel orientation in this study.

Blood mobilization during hemorrhage. In our study, 4.4 ml of blood were withdrawn on the average to reduce arterial pressure to 40 mmHg. On the basis of a total blood volume of 5.5% of body weight (1) and an average body weight of 254 g, the rats in this study had an average total blood volume of ~14.0 ml. Therefore, whereas the hemorrhage decreased total blood volume by over 31%, blood volume in the skeletal muscle venules decreased only 3.8%, based on the observed diameter changes. These results suggest that venules of skeletal muscle make only a modest contribution to mobilization of blood during hemorrhage. Similar findings were obtained with hemorrhage in the cat (38) where total blood volume was decreased by 40% and sartorius muscle venular diameters did not change significantly. Studies by Gray (15) reported a small venular constriction in the medium- and large-sized (>50 μm) venules of rat spinotrapezius muscle during prolonged (>1 h) hemorrhages, but quantitative diameter measurements were not reported. In our preliminary experiments, we maintained hemorrhagic hypotension for periods of 10 min without increasing venular constriction beyond that observed in this study.

Implications for vascular resistance. The primary purpose of this study was to evaluate the possible contribution of reduction in venular diameter to the increase in venous vascular resistance previously reported during reduced blood flow. This phenomenon has been observed in various tissue preparations in different species, among them the dog hindlimb (36), the cat lateral gastrocnemius muscle (6), the cat sartorius muscle (18), and the cat mesentery (9).

In an earlier study in our laboratory, we showed that decreasing the flow in the cat lateral gastrocnemius muscle by 70% increased venous resistance by 235% (6). This increase in resistance was shown to be at least partially due to the effect of red blood cell aggregation. In the present study, flow velocities in the venules dropped over 95% during pressure reduction with only a slight change (~2%) in venular diameter (see Table 1). On the basis of Poiseuille's equation, this diameter reduction alone would increase resistance <10%. Because we have previously shown that nearly 70% of the pressure drop in the venous network of the cat sartorius muscle occurs across the venules in the diameter range 25–185 μm (18), these earlier observations coupled with the present results support our hypothesis that nongeometric factors such as the flow properties of blood are the major cause of the observed increase in resistance.

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