Micropressure-Flow Relationships in a Skeletal Muscle of Spontaneously Hypertensive Rats

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SUMMARY Direct intravital microscopy was used to analyze microcirculatory changes in the exteriorized spinotrapezius muscle of spontaneously hypertensive rats (SHR). The animals were anesthetized with a mixture of chloralose-urethane, and measurements made of pressure, flow, and resistance in vessels ranging in size from 50 to 5 μm. The vascular changes in SHR were compared with matched Wistar-Kyoto (WKY) strain animals for both young (5-6 weeks old) and mature (12-13 weeks) rats. Distinctive changes in the distribution and levels of pressure, flow, and resistance were seen in the entire microvascular network during both stages of the syndrome. There was no significant increase in the resistance of the conduit arteries just proximal to the muscle proper. Blood pressure in hypertensives was brought down to normal and even below normal at the level of the capillaries and postcapillaries irrespective of the height of the pressure in the major artery supplying the muscle. The greater drop in pressure across the arteriolar branchings of the hypertensives was seen as early as at 5-6 weeks of age; this difference is much more striking in 12-13 week-old mature hypertensives. The reduction in pressure was proportionately greatest in the region of the smallest (10-15 μm) precapillaries of hypertensives. Resistance values were below normal in the confluent capillaries and postcapillaries in both young and mature hypertensives. Volumetric flow, which was marginally higher throughout the arteriolar branchings, fell below normal on the postcapillary side. Since an increased resistance developed at an early age (5-6 weeks) in all of the microvessels on the precapillary side, the suggestion is advanced that hypertension is associated with a generalized effect on the muscular arterioles below 30 μm, an effect that becomes more pronounced with time and at 12-13 weeks begins to involve larger sized arterioles (30-40 μm wide).

KEY WORDS • hemodynamics • SHR • microvascular resistance • flow arterioles • flow venules • micropressure distribution • skeletal muscle microcirculation • microcirculation • hypertension

It is commonly accepted that a progressive increase in total peripheral resistance associated with different forms of hypertension represents the key factor responsible for the sustained elevation in systemic blood pressure (BP). There is no agreement, however, as to the nature of the involvement of the microcirculation in the genesis of hypertension. Hypotheses in this regard range from a functional imbalance associated with an increase in the reactivity of vascular smooth muscle to structural modification of the arteriolar wall. The extraordinary lability of the microcirculation and the diversity of factors that can affect its behavior have made it difficult to match phenomenological observations with specific regulatory mechanisms. It has been especially difficult at the level of the microcirculation to distinguish between primary factors concerned with initiation of the syndrome and secondary homeostatic adjustments to the chronic elevation of BP.

The term “arteriole” is used in a general context that includes vessels ranging in diameter from 100 to 15 μm. A considerable number of investigators believe that the sustained hypertension in SHR is the result of a medial hypertrophy of the walls of the larger arterioles. These 75-100 μm wide vessels modulate overall peripheral resistance in line with changes in systemic BP and are primarily under the influence of the sympathetic nervous system. Other workers have concluded that the site of increased resistance during the evolution of hypertension is the smaller ramifications, the 15-20 μm arterioles that come increasingly...
under the influence of chemical mediators in line with their intimate involvement in the adjustment of local blood flow.

In recent years, intravital microscopy has developed into a highly valuable tool for defining microvascular behavior, particularly as more precise methods became available for continuous measurement of microvascular pressure and flow. In large part, application of these methods to the problem under discussion has involved a genetically determined form of hypertension in the spontaneously hypertensive rat (SHR), a selected offshoot of the normotensive Wistar-Kyoto strain (WKY) rats. Intravital microscopy has demonstrated abnormalities in microvessel distribution and wall characteristics of the rat mesentery, as well as a distortion of pressure distribution in the cat mesentery, in the rat cremaster muscle, and in the anterior gracilis muscle of the rat. Since these studies were limited to single variables, such as pressure or vessel number, the data are not sufficient by themselves to deal directly in quantitative terms with either the increase in vascular resistance or the factors responsible for the observed changes.

We elected to examine the microcirculation in a strap-like muscle on the back of the rat, the spinotrapezius, which is thin enough to be studied in rats weighing up to 300 g. The comparison included measurements in a 5–6 week-old set of SHR and WKY animals and a more mature set, 12–13 weeks old. Microvascular resistances at various sites were calculated from direct measurements of both pressure and flow in individual vessels. The data for the network as a whole were likewise analyzed in terms of pressure-flow relationships.

Glossary of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>Wistar-Kyoto strain of rats</td>
</tr>
<tr>
<td>SHR</td>
<td>Spontaneous hypertensive rats</td>
</tr>
<tr>
<td>$P_a$</td>
<td>Systemic blood pressure</td>
</tr>
<tr>
<td>$P_m$</td>
<td>Micropressure</td>
</tr>
<tr>
<td>Q</td>
<td>Microvessel flow</td>
</tr>
<tr>
<td>$aR_T$</td>
<td>Apparent segmental resistance for individual vessels</td>
</tr>
<tr>
<td>$eR_{pre}$</td>
<td>Effective precapillary resistance</td>
</tr>
<tr>
<td>$eR_{post}$</td>
<td>Effective postcapillary resistance</td>
</tr>
<tr>
<td>CI</td>
<td>95% confidence interval</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>S(t)</td>
<td>Cubic spline function</td>
</tr>
<tr>
<td>$E(\beta)$</td>
<td>Residual mean square error</td>
</tr>
<tr>
<td>p</td>
<td>Number of breakpoints</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Function of several physical quantities</td>
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<tr>
<td>$Y_1$</td>
<td>Physical variables</td>
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<tr>
<td>$y_1$</td>
<td>Estimate of physical variables</td>
</tr>
<tr>
<td>V</td>
<td>Variance</td>
</tr>
<tr>
<td>n</td>
<td>Number of cases</td>
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</table>

Methods

The SHR and WKY rats were obtained from Charles River Breeders. At 5–6 weeks of age, the average weight of the WKY rats was $140 \pm 8$ g, while age-matched SHR weighed $125 \pm 8$ g. At 12–13 weeks, the WKY weighed $223 \pm 17$ g and the SHR, $214 \pm 10$ g. The two sets of animals were thus approximately of the same weight.

Critical to the successful use of exteriorized tissues for a comparison of macro and microhemodynamics is the awareness of possible adverse effects of general anesthesia on the systemic BP. In the present studies, a more reliable measure of systemic arterial pressure was obtained prior to general anesthesia by surgical implantation of an indwelling PE 10 catheter into the lower abdominal aorta via the femoral artery. Previous studies mainly used the less reliable tail plethysmograph procedure.

The procedure is well tolerated and can be used to provide measurements of systolic, diastolic, and mean BPs for as long as 6 hours (De Lano and Zweifach, unpublished data). For purposes of the present studies, only mean systemic BPs ($P_s$) were used. Studies in the literature on SHR animals for the most part have used mixtures of chloralose and urethane (c-u) for intravital microscopy. We tested several combinations of these agents and adopted a mixture of 1% chloralose and 13.3% urethane (0.6 ml/100 g, i.p.) since, with this anesthetic modality, arterial pressure was lowered by only 15–20 mm Hg in hypertensives and 15–17 mm Hg in WKY controls. Pressures remained at that level during the 2.5 to 3 hours of microscopic observation.

After the spinotrapezius muscle had been exteriorized under c-u anesthesia, its distal end was freed from the dorsal body wall and cleared of sufficient surface connective tissue to permit visualization of the capillaries. Loose nylon sutures were used as ties to drape the muscle over a hollow transparent pedestal. The surface of the muscle was suffused with a balanced Krebs solution maintained at pH 7.4 by a bicarbonate CO, buffer and at 35°C. After surgical preparation of the muscle for microscopy, a period of 40–50 minutes was allowed to lapse before measurements were begun.

Changes in the environmental oxygen tension have been shown to affect the state of tone or constriction exhibited by the arterioles. A level of tissue oxygen between 5% and 8% (as measured by a platinum wire covered microelectrode), was found to produce the least interference with the microvessel response to a standard reactive hyperemia test. The rate of surface superfusion was then adjusted to maintain tissue $pO_2$ at this level. The exteriorized muscle was left uncovered and irrigated with a balanced electrolyte solution to permit direct measurements of micropressure. When only observations of diameter and flow were involved, the muscle was covered with a thin sheet of polyvinylidene chloride (Saran Wrap, Dow) that has a low permeability to oxygen. No differences in appearance or flow direction were observed in comparison to the usual uncovered and suffused preparations.
Three sets of microvascular measurements were made routinely: vessel diameter, red blood cell velocity, and hydrostatic pressure. A total of 124 rats were used, and 1505 individual vessels sampled.

Vessel diameter was measured continuously by an electronic image shearing method at an original magnification of X200 and a video screen projection image of X900. Red blood cell velocity was sensed by two separate diodes, and the delay in the movement of individual red blood cells or aggregates across the diode spacing was detected by an electronic cross correlator. Velocity was then averaged for 15-30 seconds from a continuous recording. No corrections were made to take into account velocity profile effects. Pressures were measured by inserting sharpened micropipettes (2 μm tip O.D.) into selected vessels and using the electronic servo-null principle to detect mean pressure. At the beginning of each experiment, a low power (X35) overview of the vascular network was recorded on Polaroid film and used to identify the site of individual measurements. Detailed photographic reconstructions were made of the microvascular bed in situ after a dilute solution of a colloidal carbon suspension (Gunther Wagner Company) was injected intravenously to help visualize all vessels, and the tissue was fixed with gluteraldehyde.

Microcirculatory Module Terminology

The structural and functional complex collectively known as the microcirculation is made up of an array of vessels ranging in size from 50-60 μm arterioles and venules down to the minute 4-5 μm capillaries. To document microvascular changes associated with the development of the hypertensive state, some set of guidelines had to be set up for identifying where a particular vessel was located within this complex network. A photograph and overlay tracing (fig. 1) are shown as an example of a typical branching pattern in the spinotrapezius muscle of the rat together with the nine branching categories used in the presentation of the data. We found it practical for skeletal muscle preparations to identify microvessels on the basis of two unmistakable landmarks, the A3 terminal arterioles (here called "transverse arterioles") and the capillaries proper. The transverse arteriole is a strikingly narrow (10-30 μm) vessel that is deployed at right angles to the parent arteriole and follows an almost straight course across the long axis of the skeletal muscle fibers.

An interesting feature of the vascular pattern in skeletal muscle is the presence of an arcading arrangement whereby 25-50 μm arterioles do not terminate by dichotomizing but interconnect head-on with a similar large arteriole. Each arteriolar arcade gives off 4-6 lateral offshoots, the transverse arterioles, that in turn branch into the capillary network proper.

Systemic Blood Pressure

The mean pressures (determined electrically on a Beckman Dynograph recorder) are significantly different for the WKY and SHR, 100 ± 12 SD mm Hg vs 119 ± 13 SD mm Hg in young animals (p < 0.001) and 102 ± 12 SD vs 150 ± 19 SD mm Hg for mature animals (p < 0.001). When a value of 105 mm Hg was
used to separate hypertensives from normotensive animals, 80% of SHR and 4% of WKY animals could be considered as hypertensives; for 12–13 week-old rats, a level of 120 mm Hg separated 98% of SHR and 13% of WKY into the hypertensive category. For the present study, WKY and SHR were treated separately, but those WKY and SHR that did not fall into the appropriate category for the age group were not used.

Data Handling

In the present study, as a first step, the dynamic variables were prescribed as being continuously distributed across the microvascular network (variables plotted vs diameter). This scatter plot or distribution was then fitted with a piece-wise polynomial or cubic spline function to obtain a smooth curve. Separate cubic spline fits were generated for the arterial and venous sides.

Since the application of a cubic spline function to our microvascular data is literally a least squares problem, representative or “predicted” values for the dynamic variables at any diameter may be calculated from the fitted curve along with their statistical properties by conventional methods. Predicted values may be compared and their confidence intervals determined by using normal distribution theory.

Such a presentation of the dynamic variables in terms of the cubic spline is particularly well suited for an analysis of the functional relationship between microvascular regions in SHR and WKY animals.

Although vessel classification based on branching order has been extensively used by other workers, it is not certain whether such a scheme necessarily indicates functional differences between these groups in view of the gradual nature of the change in morphology and wall structure. In most reports in the literature, mean values have been used to define the dynamic and structural characteristics of these branching orders on the assumption that they reflected symmetrical distributions.

We found that variables such as diameter, micro-pressure, and flow do not necessarily have a symmetrical distribution but often show a skewed distribution. Calculations of flow that involve a square of diameter can be misleading when based on mean or average values per se.

To examine and compare changes in selected vessel branching orders, we utilized a second procedural step. This involved the determination of the median value for the diameter of any given branching order. The standard procedure for determining medians and their confidence intervals as presented in Minitab Student Handbook was utilized. It can be appreciated that as the distribution becomes more symmetrical, the median approaches the mean.

The median values of diameter for any branching order were then projected onto the cubic spline fitted curves. With this projection we were able to retrieve “predicted” values for the dynamic variables of pressure, flow, and resistance along with their corresponding statistics (e.g., confidence interval, variance, and standard deviation).

All of our tabulated data therefore consist of “predicted” values of dynamic variables corresponding to the median diameter calculated for that branching order. Through utilization of the δ method that prescribes an estimation of the mean and variance of a function, we were able to calculate, from the predicted values of the dynamic variables, values for network resistance along with their variances. For comparison of median diameter values of one branch order relative to another, or of SHR to WKY, a Mann-Whitney U-test was carried out.

In the Appendix, we have outlined in greater detail the procedure used for applying this method to the present data on pressure, flow, and resistance.

In addition to a comparison of pressure-flow relationships between SHR and WKY animals, certain trends were determined by examining young (5–6 week) and mature (12–13 week) animals in each category. Instead of presenting a detailed analysis for each variable in both young and mature rats, pertinent data are given only when clear-cut differences are shown to exist between the two age groups. A glossary of abbreviations and symbols is also included.

Results

Microvessel Diameter

Table 1 lists both mean and median values for vessel diameter. The 95% confidence interval represents the range over which there is a 95% probability for finding the median. In mature SHR animals, vessel diameters are marginally increased for all of the branching orders, with the larger A1 type of vessel showing the greatest difference from normotensives. It can be seen that the magnitude of the difference in diameters between vessels in SHR and WKY varies with particular branching orders.

In young animals, A1 arterioles and all of the venules are from 3% to 33% wider in hypertensives, with the 33% increase occurring on the venous side in the larger V1 venules. It should be noted that A2, A3, and A4 arteriolar vessels in young SHR are actually narrower than in WKY preparations.

Microvascular Pressures

Micropressures (P_m) were measured directly for 757 individual vessels in 124 rats. It should be pointed out that the set of distribution plots (figs. 2–5) comparing SHR and WKY deal only with the network included between 50 μm arterioles and venules. This limitation was necessary because the largest microvessels in which both pressure and flow could be monitored consistently were in 40–50 μm arterioles (A2 type). On the
other hand, pressures could be obtained in any size microvessel, so that it was possible to include in the pressure table measurements microvessels up to 250 \( \mu \text{m} \) wide. Because of the difficulty in obtaining direct measurements of pressure in the thin capillaries without interrupting flow, the arterial and venous portions of the curves (e.g., fig. 2) are plotted separately by the cubic spline procedures. Pressures in the A4 precapillary range between 22-24 mm Hg in skeletal muscle of mature normotensives, while pressure in the immediate postcapillaries (V4) average 14-19 mm Hg, so that a reasonable estimate of capillary pressure would be between 18-20 mm Hg.

As indicated in figure 2, pressures in the A2 vessels of mature WKY averaged 63 to 68 mm Hg in contrast to 87 to 95 mm Hg in SHR, a 40% difference. Despite this difference, the pressures in the A4 precapillary and presumably in the capillaries are essentially the same in the two sets of rats, reflecting a much sharper decline in micropressure across the arteriolar branchings of the SHR animals. On the venous side of the network, the pressure distribution is quite similar in SHR and WKY. The plot shown for pressure distribution in young animals is similar to that for mature rats.

Through the use of the two-step procedure previously outlined, "predicted" values of micropressures could be tabulated for each of the successive branching orders. These data are shown in table 2 for the spinotrapezius muscle of young and mature rats. The pressures in the A1 type of arteries (ranging from 89-98 \( \mu \text{m} \) width) are about 40% below systemic arterial pressure, e.g., in the A1 type of mature WKY animals, pressures average 66 mm Hg as opposed to a mean systemic pressure of 100 mm Hg. In hypertensives, the BP in the same A1 arteries is brought down to 90-95 mm Hg (also a 40% drop). There is therefore no significant difference in vascular resistance encountered in the proximal portion of the arterial tree feeding the A1 microvessels in the spinotrapezius muscle of SHR or WKY animals.

It can be seen that, in the microvascular network proper of mature SHR, \( P_m \) values in the A1, A2, A3, V3, and V2 vessels are significantly higher than in WKY. A similar trend exists in young animals, but it is not as striking. Further evidence of the difference in micropressure values for the separate branching orders was obtained by calculating the ratio of the micropressures for SHR and WKY \( (P_m)_n/(P_m)_w \). As shown in table 2, the ratio favors hypertensive vessels for the A1, A2, and A3 branching orders, but then falls below 1.0 in the A4 precapillaries and the V4 postcapillaries. Beyond this region in the venules, hypertensive \( P_m \) values again predominate so that the ratios for V2 and V1 are comparable to A1 and A2 values. In young animals, the differences between SHR and WKY are less striking despite the fact that systemic BP is significantly higher in SHR.

### Micro vs Systemic Pressures

The discrete nature of the change in \( P_m \) in hypertensives is further reinforced by another comparison shown in table 2, where the trends in the predicted micropressures for the several branching orders are related to those for the systemic BP. If the
MATURE

Arteriole Venous

MATURE

Arteriole Venous

YOUNG

Arteriole Venous

young hypertensive SHR rats using the cubic spline function to estimate predicted values for vessels of different diameters. The curves are based on all of the measurements in a given scatter plot. In mature animals, there were 270 measurements for WKY, and 208 for SHR; in young animals, 142 for WKY and 147 for SHR. On each curve is indicated the predicted pressure values for the A2, A3, and A4 class of arterioles as well as for V4, V3, and V2 type of venules. These micro pressure values were obtained by calculating median diameter values for each of the branching orders (see table 1) and projecting onto the corresponding curve for SHR and WKY. Error bars represent standard deviation for each branching order. SHR animals have a much steeper pressure drop on the arteriolar side in both young and mature rats. The overlap on the venular side makes the difference in micro pressures values for SHR and WKY non-significant.

Figure 2. Distribution plots for micropressure. Separate continuous curves were fitted for normotensive WKY and hypertensive SHR rats using the cubic spline function to estimate predicted values for vessels of different diameters. The curves are based on all of the measurements in a given scatter plot. In mature animals, there were 270 measurements for WKY, and 208 for SHR; in young animals, 142 for WKY and 147 for SHR. On each curve is indicated the predicted pressure values for the A2, A3, and A4 class of arterioles as well as for V4, V3, and V2 type of venules. These micro pressure values were obtained by calculating median diameter values for each of the branching orders (see table 1) and projecting onto the corresponding curve for SHR and WKY. Error bars represent standard deviation for each branching order. SHR animals have a much steeper pressure drop on the arteriolar side in both young and mature rats. The overlap on the venular side makes the difference in micro pressures values for SHR and WKY non-significant.

Figure 3. Micropressure (P_m) normalized with respect to systemic blood pressure (P_s). If the hypertensive (H) and the normotensive (N) ratios were the same, the values would fall on the zero baseline. Instead, the ratios for the separate branching orders all are negative, with the exception of the A2 vessels in young animals, indicating that the P_m values in hypertension are increased proportionately less than the corresponding P_s values. The changes are essentially the same in young and mature rats, except for the larger venules.

Microvascular Blood Flow

Although individual flow rates have a similar distribution in mature SHR and WKY animals (fig. 4), localized differences are present. Flow falls off in mature animals at a uniform rate from a level of 11-12 X 10^{-8} mm^3/sec in the 40-45 µm arterioles to 0.08-0.10 X 10^{-8} mm^3/sec in the precapillaries, and to
an average level of $0.03 - 0.015 \times 10^{-6}$ mm$^3$/sec in the true capillary region. Because of the range of variation encountered in SHR and WKY, differences in flow rates for particular branching orders are not statistically significant. It should be noted that in young hypertensives flow on the arterial side tends to be below that in normotensives, although the differences here are not statistically significant.

Microvascular Resistance

Microvascular resistance values were analyzed in several ways, each of which will be discussed separately. Figure 5 presents a plot of what might be termed "apparent segmental resistance" ($aR_s$) for the individual vessels that make up the successive segments of the microcirculation. The $aR_s$ values represent the segmental resistance determined by measuring the pressure drop between an upstream ($P_i$) and a downstream ($P_s$) point in the network and relating this net pressure to the flow through that portion of the network,

$$aR_s = \frac{P_i - P_s}{Q_s}.$$

**Table 2.** Pressure Relationships in Microcirculation of Hypertensives

<table>
<thead>
<tr>
<th>Vessel type</th>
<th>Mature rat</th>
<th>Young rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat</td>
<td>Pm (mm Hg)</td>
</tr>
<tr>
<td>A1 feeding artery</td>
<td>SHR</td>
<td>91.3 ± 4.2</td>
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<tr>
<td></td>
<td>WKY</td>
<td>65.8 ± 2.2*</td>
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<tr>
<td>A2 arcading arteriole</td>
<td>SHR</td>
<td>62.7 ± 2.6</td>
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<td></td>
<td>WKY</td>
<td>43.0 ± 1.5*</td>
</tr>
<tr>
<td>A3 transverse arteriole</td>
<td>SHR</td>
<td>37.4 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>29.8 ± 1.5*</td>
</tr>
<tr>
<td>A4 precapillary arteriole</td>
<td>SHR</td>
<td>21.0 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>23.8 ± 1.1</td>
</tr>
<tr>
<td>V4 postcapillary venule</td>
<td>SHR</td>
<td>15.2 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>16.0 ± 0.5</td>
</tr>
<tr>
<td>V3 collecting venule</td>
<td>SHR</td>
<td>14.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>12.8 ± 0.6*</td>
</tr>
<tr>
<td>V2 small muscular venule</td>
<td>SHR</td>
<td>12.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>9.9 ± 0.6*</td>
</tr>
<tr>
<td>V1 small vein</td>
<td>SHR</td>
<td>7.2 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>5.1 ± 1.2</td>
</tr>
</tbody>
</table>

Pm = micropressure ± standard deviation; H = hypertensive; N = normotensive; Ps = systemic blood pressure.

*Significant difference between SHR and WKY to 0.05 level or better.
The term "apparent" is used since the $\Delta P/Q$ calculations are made under the assumption that the $P_i$, $P_a$, and $Q_a$ measurements represent values at the midpoint of each branching order. Since vessel length and diameter are factors affecting the measured pressure drop and the resulting flow rate, estimation of the absolute numerical values for resistance requires a consideration of both length and diameter. The diameter factor has been taken into account in the comparison of apparent resistances for SHR and WKY by obtaining projected values for pressure and flow at the appropriate median diameter for that branching order. There are no data on differences in length for the successive branching orders in SHR and WKY, leaving unanswered the reason for the observed differences in $aR$, between hypertensives and normotensives.

The data for segmental $aR_i$ values are shown in histogram form comparing SHR and WKY branching orders (fig. 5). In general, $aR_i$ values increase in both groups on the arterial side since the hierarchy of vessels become successively narrower, whereas on the venous side, $aR_i$ values fall off as the vessels become wider through repeated confluences. However, the arterial vessels in SHR have a consistently higher resistance at each branching order beyond the A1 vessels, with the greatest difference occurring in the A4 precapillary region.

In young SHR, the A3 type are the most proximal arterial vessels in which $aR_i$ values are significantly increased, whereas the trend for an increased resistance in more mature SHR becomes clearly evident in the A2 arcading arterioles. Venular resistances show a similar trend in both WKY and SHR beds. Resistances are actually lower in the V4 and V3 postcapillaries of mature SHR than in WKY controls.

Further insight into the altered resistance relationships was obtained by comparing the predicted resistance values from the cubic spline curves for the successive branching orders of SHR and WKY networks. Ratios of segmental $aR_H$ and $aR_N$ values (table 3) provide a convenient quantitative index of the segmental change in resistance during the 5th and 13th weeks of the hypertensive state. The resistance ratio increases from near equivalence (0.87) in the A2 region up to 2.69 in the A4 precapillary region of mature animals. On the venous side, the ratio falls below 1.0, indicating that V4 postcapillary and V3 resistances are actually lower than normal in hypertensives. It should be noted that the median diameter for the V4 postcapillaries is significantly wider in SHR than in WKY, 9 $\mu$m vs 7 $\mu$m (see table 1), a fact that could account for the reversed trend in resistance in this region of the network (61.9 for WKY vs 31.2 for SHR). A similar trend for resistance to fall below that in the postcapillaries of normotensive animals is seen in young SHR.

Microcirculatory Network Resistance

In the previous section, pressure-flow relationships were detailed for individual vessels in different loca-
TABLE 3. Segmental Resistance in Microcirculation of Hypertensives

<table>
<thead>
<tr>
<th>Vessel type</th>
<th>Rat</th>
<th>Mature rat</th>
<th>Young rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Flow (10^-3 mm/sec)</td>
<td>Apparent resistance (AR) (mm Hg/10^-3 mm/sec)</td>
</tr>
<tr>
<td>A1 feeding</td>
<td>SHR</td>
<td>12.4 ± 2.9</td>
<td>2.23 ± 0.676</td>
</tr>
<tr>
<td>artery</td>
<td>WKY</td>
<td>8.93 ± 1.4</td>
<td>2.55 ± 0.499</td>
</tr>
<tr>
<td>A2 arcading</td>
<td>SHR</td>
<td>1.17 ± 0.11</td>
<td>21.52 ± 3.700</td>
</tr>
<tr>
<td>arteriole</td>
<td>WKY</td>
<td>1.19 ± 0.17</td>
<td>11.00 ± 2.377*</td>
</tr>
<tr>
<td>A3 transverse</td>
<td>SHR</td>
<td>0.11 ± 0.01</td>
<td>149.1 ± 31.95</td>
</tr>
<tr>
<td>arteriole</td>
<td>WKY</td>
<td>0.11 ± 0.01</td>
<td>55.45 ± 17.64*</td>
</tr>
<tr>
<td>A4 precapillary</td>
<td>SHR</td>
<td>0.093 ± 0.012</td>
<td>31.18 ± 17.82</td>
</tr>
<tr>
<td>arteriole</td>
<td>WKY</td>
<td>0.063 ± 0.009*</td>
<td>61.9 ± 15.22</td>
</tr>
<tr>
<td>V4 postcapillary</td>
<td>SHR</td>
<td>0.31 ± 0.03</td>
<td>1.93 ± 3.43</td>
</tr>
<tr>
<td>venule</td>
<td>WKY</td>
<td>0.28 ± 0.04</td>
<td>1.14 ± 3.23*</td>
</tr>
<tr>
<td>V3 collecting</td>
<td>SHR</td>
<td>2.26 ± 0.31</td>
<td>0.796 ± 0.483</td>
</tr>
<tr>
<td>venule</td>
<td>WKY</td>
<td>3.66 ± 0.5</td>
<td>0.792 ± 0.255</td>
</tr>
</tbody>
</table>

AR = apparent resistance individual vessels; H = hypertensive; N = normotensive.

*Significance to 0.05 level or better.

TABLE 4. Cross Sectional Resistance for Microvascular Network (Mature Rat)

<table>
<thead>
<tr>
<th>Input vessel type</th>
<th>Flow (10^-5 mm/sec)</th>
<th>Matching vessels</th>
<th>Effective microvascular resistance (eR) (mm Hg/10^-5 mm/sec)</th>
<th>(eR)_H (mm Hg/10^-5 mm/sec)</th>
<th>Effective precapillary resistance (mm Hg/10^-5 mm/sec)</th>
<th>(eR)_H Xpre</th>
<th>Effective postcapillary resistance (mm Hg/10^-5 mm/sec)</th>
<th>(eR)_H Xpost</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2 SHR</td>
<td>10</td>
<td>32.2</td>
<td>4.92 ± 0.271</td>
<td>1.44</td>
<td>3.62 ± 0.33</td>
<td>1.95</td>
<td>0.74 ± 0.12</td>
<td>1.06</td>
</tr>
<tr>
<td>WYK</td>
<td>10</td>
<td>38</td>
<td>3.40 ± 0.16*</td>
<td>1.36</td>
<td>1.86 ± 0.19*</td>
<td>1.36</td>
<td>0.70 ± 0.08</td>
<td>1.06</td>
</tr>
<tr>
<td>A2 SHR</td>
<td>5</td>
<td>24.1</td>
<td>7.47 ± 0.47</td>
<td>1.32</td>
<td>5.60 ± 0.60</td>
<td>2.03</td>
<td>0.76 ± 0.20</td>
<td>1.22</td>
</tr>
<tr>
<td>WYK</td>
<td>5</td>
<td>29.1</td>
<td>5.66 ± 0.27*</td>
<td>2.12</td>
<td>2.76 ± 0.35*</td>
<td>2.12</td>
<td>1.22 ± 0.10*</td>
<td>1.22</td>
</tr>
<tr>
<td>A3 SHR</td>
<td>1.0</td>
<td>13.3</td>
<td>22.60 ± 2.0</td>
<td>1.22</td>
<td>15.7 ± 3.0</td>
<td>2.90</td>
<td>1.31 ± 1.1</td>
<td>0.28</td>
</tr>
<tr>
<td>WYK</td>
<td>1.0</td>
<td>12.9</td>
<td>18.45 ± 1.6*</td>
<td>1.22</td>
<td>15.41 ± 1.8*</td>
<td>2.90</td>
<td>4.64 ± 0.8</td>
<td>1.17</td>
</tr>
<tr>
<td>A3 SHR</td>
<td>0.3</td>
<td>9.4</td>
<td>49.0 ± 7.5</td>
<td>1.05</td>
<td>28.6 ± 1.0</td>
<td>3.3</td>
<td>1.67 ± 3.7</td>
<td>0.17</td>
</tr>
<tr>
<td>WYK</td>
<td>0.3</td>
<td>9.0</td>
<td>46.5 ± 4.0</td>
<td>1.05</td>
<td>8.67 ± 5.2*</td>
<td>3.3</td>
<td>9.87 ± 2.3*</td>
<td>0.17</td>
</tr>
<tr>
<td>A4 SHR</td>
<td>0.11</td>
<td>6.8</td>
<td>64.3 ± 12.6</td>
<td>1.05</td>
<td>3.62 ± 0.33</td>
<td>1.95</td>
<td>0.74 ± 0.12</td>
<td>1.06</td>
</tr>
<tr>
<td>WYK</td>
<td>0.11</td>
<td>6.7</td>
<td>85.4 ± 10.7</td>
<td>1.05</td>
<td>4.92 ± 1.2</td>
<td>4.92</td>
<td>0.70 ± 0.08</td>
<td>1.06</td>
</tr>
<tr>
<td>A4 SHR</td>
<td>0.095</td>
<td>6.4</td>
<td>58.9 ± 14.6</td>
<td>0.63</td>
<td>28.6 ± 1.0</td>
<td>3.3</td>
<td>1.67 ± 3.7</td>
<td>0.17</td>
</tr>
<tr>
<td>WYK</td>
<td>0.095</td>
<td>6.4</td>
<td>93.3 ± 11.8</td>
<td>0.63</td>
<td>8.67 ± 5.2*</td>
<td>3.3</td>
<td>9.87 ± 2.3*</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*Statistically significant to 0.05 level.
†Standard deviation.
eR_T = total resistance; H = hypertensive; N = normotensive.
week-old categories were essentially of the same weight.

The A2 type of arteriole was taken as the input reference for our calculation of total network resistance ($eR_T$). Then, by using the flow distribution cubic spline curve to identify a venular vessel with the same flow as in the A2 arteriole, we were able to measure the total resistance across an entire microcirculatory module (table 4). For each branching order, two different flow rates were used to calculate $eR_T$ values. As shown, the size of the bed for which network resistance values were calculated was decreased stepwise by selecting vessels with lower flow rates for each of the successive branching orders.

It can be seen that the set of figures at the bottom of table 4 (where flow rate is equal to $0.9 \times 10^{-4} \text{mm}^3/\text{sec}$) reflects the operational characteristics of the true capillary portion of the network. Since resistance and vessel diameter have a diametrically opposite relationship, $eR_T$ is increased more than tenfold as the percentage of the vessels making up the intervening network favors the minute capillaries. When the A2 arcading arterioles and A3 transverse arterioles are used as the boundary reference, $eR_T$ values are substantially higher in SHR. On the other hand, the situation is reversed when A4 precapillaries are used as an input reference point and $eR_T$ values in SHR are lower than in WKY controls (59 vs 93 mm Hg/10$^{-4}$mm$^3$/sec).

The differences in the $eR_T$ trend for WKY and SHR are also expressed in table 4 as a ratio $([eR_T]_{WKY}/[eR_T]_{SHR})$. Thus, total effective resistance across the entire A2 to V2 microvascular network is 44% higher in hypertensives but only 5% higher in that portion of the network bounded by the 9-10 $\mu$m precapillaries and their flow-matched venules. Finally, across the capillary portion of the network, $eR_T$ in mature SHR is actually 37% below normal.

The effective network resistance can be broken down into pre- and postcapillary portions by using the A4 arterioles as the reference point for precapillary resistance and the V4 postcapillaries for the calculation of postcapillary resistance. Precapillary resistance values are from 1.9 to 2.03 times higher in SHR when the network included between A2 arcades and V2 venules is used, whereas, when the A3 arterioles are used as input vessels, precapillary resistance values are 2.9 to 3.3 times higher.

Examination of network postcapillary resistance values ($eR_{post}$ in table 4), reveals an interesting trend. The difference in $eR$ values between SHR and WKY becomes progressively greater as the size of the network is narrowed. At the point where 15 $\mu$m venules are used to match an input flow rate of $0.3 \times 10^{-4} \text{mm}^3/\text{sec}$, postcapillary $eR$ is 9.87 for WKY controls as opposed to only 1.67 for SHR, a sixfold difference. Obviously, these data reflect a substantial modification in the intervening network in hypertensive animals that is most pronounced in the smaller vessels of the network.

The pre:postcapillary resistance ratios listed in table 4 show a progressive increase (almost fourfold) for different segments of the microvascular network of SHR, being highest when the network consists only of the precapillaries, capillaries, and postcapillaries. This trend is in direct contrast to that observed in WKY which show an opposite trend and decline substantially as the larger vessels are excluded. The actual increase in the pre:postcapillary resistance ratio in SHR is therefore the resultant of a progressively higher precapillary resistance and concurrently lower postcapillary resistance.

### Discussion

Analyses of microvascular behavior have been made either on the basis of the performance of the network as a whole, or by subdividing the vascular array into discrete segments depending upon the availability of individual measurements. Most of the intravital studies in the literature have adopted this latter approach. Although classification of microvessels into groups presumed related to the basis of branching order is useful for establishing general trends in the hypertensive state, the range of vessel sizes and types and the continuous vasomotion exhibited by the microvasculature, make it necessary to incorporate other criteria for a more detailed analysis of the problem. In the present study on the spinotrapezius muscle, instead of working only with averaged values for groups of vessels, a more accurate representation of the distribution of basic hemodynamic variables (pressure, flow, and resistance) was obtained by using a cubic spline function to fit a continuous curve to all of the individual measurements. In addition, the calculation of median diameter values for each of the recognized branching orders allowed us to project these values onto the continuous pressure or flow distribution curves and thereby obtain the corresponding average or "predicted" numerical value for each of the successive branching orders of the microcirculation. Data of this kind obtained separately for WKY and SHR animals show clearcut changes in both the early and late stages of hypertension.

### Basis of Hypertension

One of the commonly accepted hypotheses of hypertension equates the progressive rise in systemic BP with an increase in the reactivity of vascular smooth muscle either through the intervention of vasoactive chemical factors, or the activation of neurogenic mechanisms. Support for the first of these concepts derives primarily from studies on renal hypertension, although recent intravital studies question an increased sensitivity to norepinephrine in both SHR and renal hypertension. Evidence for the latter concept is based in large part on cardiovascular hemodynamics in spontaneously hypertensive rats. Bohr believes that disproportionate changes in venous vessel tone could lead to increased venous
Another highly regarded concept favors structural reorganization of the arteriolar wall as a major circumstance that becomes fixed with time and limits the ability of these vessels to undergo vasomotor adjustments.\textsuperscript{57, 58} Still other studies on skeletal muscle\textsuperscript{39, 40} have raised the possibility that, in addition to intrinsic modifications in individual components of the microvascular network, the early stages of hypertension are associated with a reduction of the number of small vessels that are actively perfused, primarily the terminal arterioles (A3) and their ramifications. In contrast, during the later stages of the hypertensive syndrome in SHR, the number of venules appears to be increased by as much as 60%\textsuperscript{38}.

In the present studies, the elevation of systemic BP in SHR was found to be associated not only with localized increases in the resistance of individual microvessels but with an increase in the collective resistance of the microvascular network as a whole. The pervasive nature of the redistribution of microvascular resistance is apparent even in 5-week-old SHR animals.

Separate calculations of precapillary and postcapillary resistance bring out another interesting difference between normotensive and hypertensive vascular beds. At the time that the effective precapillary resistance is being increased several fold, postcapillary resistance across the collecting venules and muscular venules is much lower in hypertensives. Dusseau and Hutchins,\textsuperscript{46} on the basis of vessel counts and the effects of treatment with a $\beta_2$ agonist, have obtained evidence in SHR suggesting that the number of small venules is almost doubled in cremaster muscle preparations. The net effect of this type of alteration could be to reduce overall venous resistance. Such a situation is in line with the reduced postcapillary eR values cited in the present study.

Altered Distribution of Micropressures

The pressure measurements in the present studies by themselves demonstrate the selective nature of the modifications of the microvascular bed in hypertension. Pressure does not fall off at a uniform rate across the arteriolar branchings, but the decline is sharply exacerbated in the region of the A4 precapillaries. This latter phenomenon is further exaggerated in hypertensive vascular beds as early as 4 to 5 weeks of age. The data available to us at this time do not permit identification of the precise structural or functional mechanisms that may be implicated. The fact that micropressures continue to be adjusted selectively in the various branching orders in the presence of an elevated systemic pressure suggests that these adjustments may be secondary in nature. Obviously such an inference is only one of many possibilities.

Our findings on the spinotrapezius muscle are not fully compatible with a number of earlier studies on the cremaster muscle in SHR\textsuperscript{8, 11, 44} from which it was concluded that micropressures are increased by a constant fraction in direct proportion to the elevation in systemic BP throughout the network, with the exception of the arteriolar vessels in the A2–A3 region. Since the comparison between micro and systemic pressures in the studies cited above was formulated solely from average values for the successive branching orders, the authors were not in a position to analyze either the basis for this difference in the behavior of the A2 and A3 arterioles, or the extent to which the immediately contiguous vessels on the upstream and downstream side may have been involved.

Age Factor

The distribution of micropressures within the terminal vascular network of young hypertensives differs in several respects from that in mature hypertensives. For example, in 5–6 week-old SHR, the segmental resistance for the A2 type of arcading arterioles is increased by almost 80% above that of WKY vessels, whereas in 12–13 week-old animals, segmental values for A2 vessels are essentially the same. During the two periods of observation, systemic BP is elevated by 30%–40% in hypertensives and yet A3 and A4 micropressures are adjusted to normal levels. Apparently, the components of the microvascular network are either passively or actively altered during the 7–8 week interval separating the two sets of observations. The basic mechanisms involved in this readjustment remain conjectural.\textsuperscript{44}

Microvascular Resistance

Blood is moved through the microcirculation along tubes of changing diameter and length, across branching configurations with different geometrical constraints, and with variable red cell hematocrits in the successive subdivisions of the network. Hence, changes in overall resistance can be the end result of multiple factors, including the number of actively perfused vessels, as well as the relative number of in-series and in-parallel circuits.

It is therefore highly significant that, despite substantial differences in the pressure driving the blood across the successive segments of the microvascular network, volumetric flow was found to fall off at a comparable rate in normotensive and hypertensive animals. In view of the close interdependence of pressure and flow, hypertension must be associated with an appropriate modification in the resistance encountered at the successive levels of the arterial microcirculation, especially from the A1 type arterioles down to the A4 precapillaries.

The fact that the entire hierarchy of arteriolar vessels show some increase in segmental resistance in hypertension strongly suggests that a common denominator is involved, a particularly plausible one being structure/function changes in vascular smooth muscle. Since our findings in both 5–6 week-old and
12-13 week-old SHR indicate that the greatest increase in resistance occurs in the region of the smallest (A3, A4) arterioles, the data do not support the early involvement of a medial hypertrophy, a phenomenon that presumably occurs in the larger (A1, A2) arterioles where pressure is significantly elevated.

Calculations of precapillary network resistance across the entire hierarchy of arteriolar vessels show an almost twofold increase in SHR animals. The principal locus for this increase in resistance would appear to be the A3 and A4 precapillary vessels, which in mature SHR show $r_{pre}$ values three times greater than in normotensives.

In view of the demonstrated increase in overall vascular resistance during hypertension, some narrowing of the arterioles might have been anticipated. Our observations in the spinotrapezius muscle, as well as the data of Hutkins and Darnell, Bohlen et al., and Henrich and Hertel in the cremaster muscle indicate that luminal diameters of the A1, A2, and A3 arterioles are in fact, on the average, slightly larger in hypertensive vascular beds. A direct comparison between vascular diameters in SHR and WKY is difficult to achieve, however, in view of the range of vessel diameters within branching orders.

Consideration should be given not only to the fact that the absolute level of the systemic pressure is elevated to a different degree, depending upon the age at which the microvascular measurements are made, but to the fact that the arteriolar network of vessels has been exposed to an above normal transmural pressure and wall tension for different periods of time. This latter feature is of singular importance in analyzing the structural modifications that develop secondarily during hypertension.

**Rarefaction**

Bohlen et al. and Hutchins and Darnell believe that a substantial portion of the increased resistance in spontaneously hypertensive rats is due to a rarefaction, as a consequence of which fewer A3 and A4 arteriolar branches were perfused. Although the measurements made in the present studies cannot provide a definitive answer to the question of numbers of perfused vs unperfused microvessels, they favor another alternative, i.e., that distinctive changes in all of the muscular microvessels contribute to the higher resistance on the precapillary side of hypertensive beds. The fact that segmental resistance values are based on direct measurements of pressure and flow in open perfused vessels weakens the argument that rarefaction per se is the major factor affecting overall resistance in mature SHR.

The A2, A3, and A4 vessels show a modest narrowing in young SHR but not in mature SHR, as would be expected if a heightened smooth muscle tone had been responsible for the increase in resistance. Among the remaining possibilities are an increase in vessel length, further restriction of flow across junctional branching configurations, and a change in vessel hematocrit. These factors have not as yet been examined systematically in relation to hypertension.

**Cause and Effect**

The intriguing feature of the measurements in the present studies is the demonstration that by the time the blood reaches the exchange portion of the hypertensive microvascular bed, both pressure and flow have been brought within the normal range irrespective of whether the arterial BP is elevated by 20-25 mm Hg in young hypertensives or by 40-50 mm Hg in older hypertensives. Such observations can be interpreted to indicate the operation of some type of local adjustment in the region of the terminal arterioles and precapillaries acting to counter the effects that an elevated BP would have on blood-tissue exchange. This issue of whether the microvascular alterations observed in hypertensive animals are primary or secondary manifestations is difficult to resolve because of the rapidity with which local adjustments can be evoked. On the other hand, the fact that altered microvascular pressure-flow relationships not only appear as early as 5-6 weeks of age but become more pronounced after the syndrome has evolved for 7-8 weeks is suggestive of a causal relationship.

**Conclusions**

The following generalizations can be drawn from the present studies on the spinotrapezius muscle:

1. Changes occur in all of the microvessels including the venules during the development of hypertension.
2. A variable but selective effect on vascular smooth muscle would seem to be indicated on the precapillary side.
3. The fact that the nonmuscular capillaries and postcapillaries show a reduction in resistance indicates further alteration in structure and/or patterns of flow in the network distal to the precapillary segment.
4. A comparison of microvascular pressure, flow, and resistance in young and more mature hypertensives shows differences in the severity of the change but not in direction.
5. The distinctive changes in resistance observed in young, 5-6 week-old hypertensives are uniformly exacerbated in older, 12-13 week-old rats but to a varying degree in the successive segments of the microcirculatory tree.
6. There is evidence of a retrograde change with time, since early in hypertension the increased segmental resistance involves only the 25 μm arterioles and their branches, whereas later in the syndrome the larger 40 μm arterioles are also affected.
Appendix

Data Handling

CUBIC SPLINES have been widely used as a flexible set of functions for curve fitting. The data are divided into segments and all the segments are fitted simultaneously, using a least square method. The advantage of such an approach is that there is no assumption that the curve is of any particular parametric form.

A cubic spline function can be defined as a piecewise polynomial of degree 3 for which the pieces are constrained to join smoothly at each breakpoint. Given breakpoints at \( t_1 < t_2 < \ldots < t_p \) (\( p = \) number break points), the spline is a cubic polynomial on each region \((t_i, t_{i+1})\) that is twice continuously differentiable at each of these points. Each cubic polynomial is determined by four parameters \((a_0, a_1, a_2, a_3)\) of \( a_0 + a_1 t + a_2 t^2 + a_3 t^3 \), but the differentiability constraints imply that there are \( p + 2 \) independent parameters.*

There are a number of ways to give parameters to cubic splines; one of the most convenient is via the B-spline approach. B-splines are defined precisely in DeBoor.** Any spline function \( S(t) \) with breakpoints \( t_1 < t_2 < \ldots < t_p \) can be represented as

\[
S(t) = \sum_{j=0}^{p+2} B_j(t) \beta_j
\]

where the \( \beta_j \)'s are coefficients of the B-splines, \( B_j \). The derivative of \( S(t) \) is simply

\[
S'(t) = \sum_{j=1}^{p+2} \beta_j B_j'(t)
\]

To fit data \( y_i, (i = 1, \ldots, n) \) to a spline function with given breakpoints by least squares, one minimizes

\[
E(\beta_1, \ldots, \beta_{p+2}) = \sum_{i=1}^{n} \left[ y_i - \sum_{j=1}^{p+2} \beta_j B_j(t_i) \right]^2.
\]

This is a linear least squares problem. When \( n \), the number of observations, is large relative to \( p \), the number of breakpoints, the covariance matrix can be calculated from the fitted coefficient, \( \beta_j \). From the covariance matrix, the variance and standard deviation at each "predicted" value can be determined by standard classical procedure.*** The entire procedure is analogous to fitting data by a least squares to a polynomial.

The first step, then, in fitting a spline function is to determine the number and location of the breakpoints. As a rule of thumb, the number of breakpoints should be as small as possible while still adequately fitting the data, and it is helpful to locate the breakpoints in regions where the function is changing rapidly. More systematically, the breakpoints may be located so that the residual sum of squared deviations from the fit is minimized. More extensive discussion may be found in DeBoor* and Wold."***

An example of an application of this method to the microcirculatory data is shown in figure 6 for the micropressure measurements. The original data for mature hypertensives are shown as separate points and cover vessels ranging in diameter from 120 \( \mu m \) arterioles, precapillaries, postcapillaries, and 120 \( \mu m \) venules. Depending upon the number and location of breakpoints, different shaped curves can be obtained ranging from a straight line to an irregular curve that goes through all the data points. The procedure adopted for this study was designed to obtain a smooth-fitting curve that is a monotonically decreasing function and has the least sum of squared deviation from the data points. Such a curve fitting is shown in the figure, with the arteriolar and venous ends of the network treated separately. Points lying on the curve represent "predicted" values for various sized microvessels for which a zone of confidence can be calculated. A rectangular area is blocked out to show the portion of the network that is displayed in our figures 2 through 6, for which both pressure and flow measurements were available.

For the pressure distribution shown and for all the cubic spline fits presented in this paper, three knots were found to be sufficient for approximating the data. In choosing the position of the knots, the two endpoints are fixed, and the location of the middle knot is moved along the diameter axis until the fitted curve satisfies the monotonically decreasing criteria and has the least sum-squared error. The above procedure was written as a Fortran subroutine by J. Fahle at the UCSD Computer Center. It is a version of the cubic spline subroutine described in DeBoor's book,* modified by adding the calculation for variances along the fitted spline curve. All computations were carried out on the Burroughs 7800 computer.

\( \delta - \) Method

In determining resistance values and their variances as a function of pressure and flow, the \( \delta \)-method (an estimation of the mean and variance of a function) was used.** Given a function \( g(Y_1, Y_2, \ldots, Y_m) \), where \( Y_i \) are unbiased variables, the variance of \( g(Y) \) can be calculated by

\[
\sigma^2 g(Y) = \sum_{i=1}^{m} \left( \frac{\delta g}{\delta Y_i} \right)^2 \sigma^2 Y_i
\]

where \( Y_i \) is the estimate of \( Y_i \), with the only assumption being that \( Y_i \) is independent.

References

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Micropressure-flow relationships in a skeletal muscle of spontaneously hypertensive rats.
B W Zweifach, S Kovalcheck, F De Lano and P Chen

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