Relationship between microvascular blood velocity and pressure distribution

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MAYROVITZ, HARVEY N., RONALD F. TUMA, AND MARY P. WIEDEMAN. *Relationship between microvascular blood velocity and pressure distribution.* Am. J. Physiol. 232(4): H400–H405, 1977 or Am. J. Physiol.: Heart Circ. Physiol. 1(4): H400–H405, 1977. – Red blood cell velocity and diameter were measured in vessels of the wing of the unanesthetized bat (*Myotis lucifugus*) from the supplying artery to the capillaries. These data were used to determine the manner in which velocity, shear rate, volume flow, and blood pressure depend on the vessel's hierarchical position within the vascular network. The results show that velocity decreases in an almost linear fashion as the capillary is approached but that the shear rate increases as one progresses distally from the supplying artery. Blood volume flow was found to decrease as an exponential function of the branching order. Comparison with available data in some animal species, including man, indicates some agreement in capillary velocity, although significantly lower values have been reported in some preparations. Using a method whereby blood pressure distribution could be obtained from anatomical data and center-line blood velocity, the rheologic alterations accompanying consecutive vessel branching were deduced and found to be in good agreement with data available in the literature.

microcirculatory hemodynamics; blood flow; shear rate; platelet aggregation; leucocyte adherence; Little Brown Bat; viscosity

THE RELATIONSHIP BETWEEN blood pressure and blood flow in the microcirculation has been studied primarily in single vessels (6, 8, 14, 15). Recent developments in techniques that make it possible to measure both blood pressure and velocity in microvessels in vivo have resulted in information regarding pressure and flow within an entire vascular bed (7, 10, 11, 29, 35). In addition, new methods have been introduced for interpretation and analysis of the data so obtained (9, 16, 20–22). Pressure and velocity measurements have been made in various vascular beds that can be observed directly through the microscope in the living animal (3, 12, 17, 24, 25, 34, 36). However, with the exception of the rabbit ear chamber and the bat wing preparation (36), anesthesia and surgical intervention are required for microscopic observation of the blood vessels.

In view of recent evidence that even mild trauma and tissue exposure may dramatically alter blood flow within the microvascular field (28), it seemed that a study of hemodynamic characteristics obtained from a vascular bed in an animal not subjected to anesthesia or surgery would provide important data. The present study was undertaken to measure velocity in consecutive branches of vessels in the wing of the Little Brown Bat (*Myotis lucifugus*) from a major artery to the capillary network to determine pressure distribution through velocity and diameter measurements within a total vascular bed.

METHODS

Experimental. The bat wing is prepared for microscopic observation as described previously (32). Briefly, the animal is placed in a tubular chamber and its wing extended over a large optical flat and the wing is held in position by small spring-loaded clips. A small region of epithelium is peeled off to enhance visibility. The denuded area was covered with Normosol (Abbott Laboratories) over which was placed a small cover glass. In this manner all levels of vessels from main artery to collecting vein are available for microscopic observation at magnifications up to ×1,200.

The prepared animal was placed on the stage of an American Optical trinocular microscope. An eyepiece micrometer was used for vessel diameter measurements. An overall magnification of ×1,200 was used in all experiments and inside diameters recorded in all vessels in which velocity was measured. The image of the vessel under observation is projected onto a viewing screen by means of a right-angle prism inserted into the trinocular barrel. Red blood cell velocity measurements are performed in a manner similar to that reported previously (11). Briefly, two matched phototransitors are inserted into small slits in the viewing screen so that they are positioned in the center of the projected image of the vessel in which velocity is to be measured. The phototransitors are aligned parallel to the vessel axis. The electrical signals obtained from the sensors represents the instantaneous optical density at two axially separated vessel sites. After preamplification of these signals, on-line velocity determinations are obtained by cross correlating the up- and downstream optical signals using a velocitometer (IPM; San Diego). This device computes the time delay between up- and downstream optical signals and, together with a calibration factor for the effective axial separation of the sensors, produces an output voltage representing the red blood cell velocity.

Calibration and reliability of the velocity tracking system used in these experiments have been verified by comparing its output with that obtained *1*) from a Hew-

lett-Packard model 3721A correlator when small tubing of known diameter was perfused with blood at a known flow rate, *2*) by electronic simulation signals of known time delay, and *3*) by moving a calibrated microscope stage micrometer at a known velocity.

For purposes of identification, the main artery entering the wing is designated as *order 0*, direct branches of it as *order 1*, branches of *order 1* as *order 2*, and so on. Velocity measurements were made in a total of 17 animals. In eight of these, velocity and diameter measurements were made in each of the consecutive branching orders from zero order to capillaries. In the remaining trials velocity determinations in a given animal were restricted to two, three, or four branching orders. In determining the velocity distribution, these data have been lumped according to their appropriate branching order. All velocities reported are directly recorded center-line velocities, which may be corrected for optical artifact using the method of Baker and Wayland (1). Average velocities, as used in the calculation of wall shear rate and volume flow, are taken as one-half the uncorrected center-line velocity.

Analytical. The velocity (v) and diameter (D) are used to calculate the pressure distribution from zero-order artery through capillaries by using a parameter which is proportional to the pressure gradient as deduced from the well-known Poiseuille relationship. When applicable this relationship (*equation 1*) states that the pressure gradient ($\Delta P/\Delta L$) may be determined from knowledge of mean velocity ($\bar{v}$), vessel diameter, and blood viscosity (η).

$$\Delta P/\Delta L = 32\eta\bar{v}/D^2 \qquad (1)$$

Direct application of this equation in vivo is complicated by many factors including the viscosity dependence on local hematocrit (13), shear rate (26), and rheologic factors relating to branching (2, 4, 23). In addition the relationship between measured center-line velocity (v) and the required mean velocity ($\bar{v}$) is dependent on the velocity profile which itself depends on the branching order.

Letting x represent the set of variables upon which viscosity depends and α represent the relationship between mean velocity and measured velocity, the above is concisely stated as

$$\eta = F(x) \qquad (2)$$

$$\bar{v} = \alpha v \qquad (3)$$

in which α itself is dependent on the local velocity profile. Since it is not possible to either simultaneously measure all of the variables (x) or routinely obtain the precise velocity profile for each of the consecutive branching orders, it is useful to define a parameter (P′) which is proportional to the pressure gradient and equal to quantities that may be easily and directly measured. For want of a better term this parameter is called the modified pressure gradient (MPG) and is given by *equation 4*.

$$P' \equiv \frac{\Delta P/\Delta L}{32\,\alpha\eta} = \frac{v}{D^2} \qquad (4)$$

To use the MPG to estimate pressure distribution, the measured values of v/D^2 for each branching order are multiplied by the average lengths of each vascular segment as reported by Wiedeman (30). The fraction of the pressure lost across each of the consecutive segments is then obtained from the ratio of that segment's length, the MPG product, to the sum of all such products from supplying artery to postcapillary venule inclusive. Based on the data of Wiederhielm and Weston (34), the absolute pressure distribution is calculated using mean pressure in the 0-order supplying artery and small vein of 90 and 10 mmHg, respectively.

RESULTS

The velocity and diameter measurements for each branching order are shown in Fig. 1. The velocity distribution obtained conforms to the classical concept of a decreasing linear velocity from larger to smaller vessels. Generally this condition prevails but occasionally velocities in a parent vessel and its branch were found to be the same. In some cases the velocity was greater in the branch vessel. This reversal in the general trend was more prevalent in the branching vessels with the larger diameters and probably reflects the state of the vasculature distal to the measuring site.

Using the simultaneous determinations of vessel diameter and velocity, wall shear rate was calculated for each branching order as shown in Fig. 2. The trend for increasing shear rate as the capillary network is approached reflects the disproportionate decrease in vessel diameter with respect to velocity for consecutive branching orders. Shear rates peak at the 3rd-order

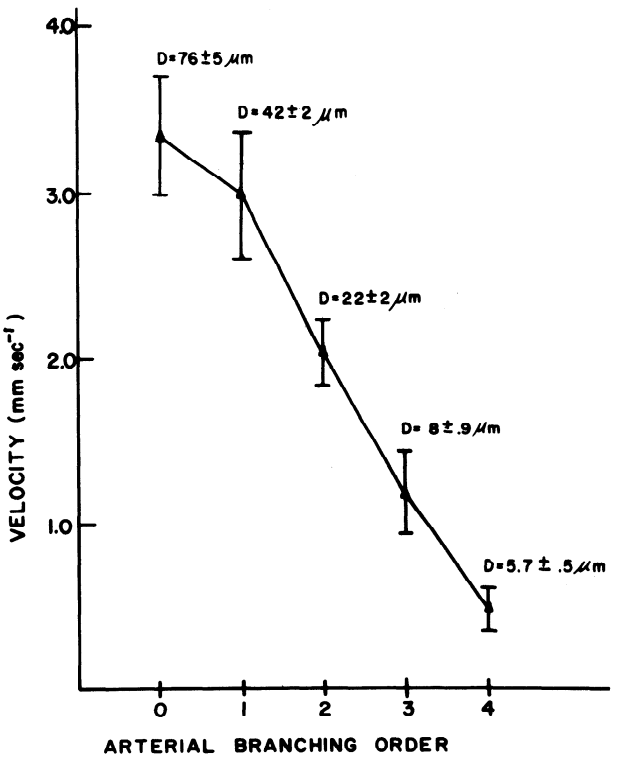

FIG. 1. Red blood cell velocity in consecutive branches of bat wing. Bars represent SE. Values above bars are vessel diameters in micrometers (mean ± SE).

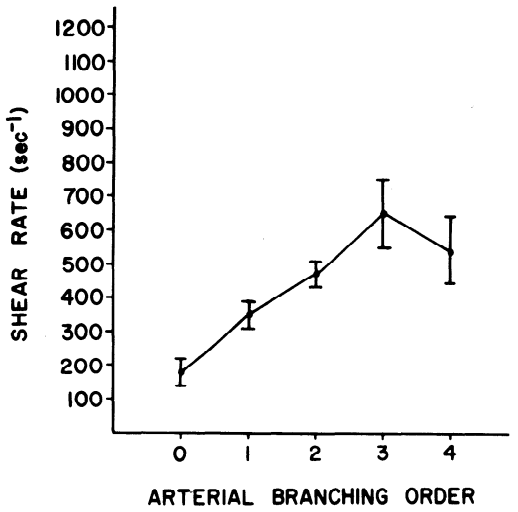

FIG. 2. Shear rate as a function of arterial branching order. Bars represent SE.

branching level and are approximately 4 times larger than in the main artery of the wing.

In order to estimate relative flow distribution, the flow in each vascular branching order is computed as the product of mean velocity and vessel cross-sectional area. In Fig. 3 flow within each consecutive branching order is presented as a percent of the flow in 0-order supplying vessels. The approximate linear dependence as displayed on the semilogarithmic representation implies a flow distribution roughly exponentially dependent on the branching order. About 0.08% of the total flow and 0.28% of the 1st-order flow is distributed to each of the terminal arterioles and capillaries. The latter flow fraction is approximately 1.5 times larger than would be predicted solely on the basis of anatomical data (30).

Using the velocity and diameter data for consecutive branching orders, the MPG is calculated and expressed in Fig. 4 relative to the MPG in the supplying artery (0-order vessel). The MPG increases with increasing branching order with the largest change in the normalized MPG occurring in the transition from 2nd- to 3rd-order vessels. Little difference is seen between 3rd and 4th order. Gradients in the 3rd-order vessel are nearly 27 times greater than in the supplying artery and almost 9 times larger than the 1st-order vessel, as shown in Fig. 5. A comparison between the normalized MPG in the wing and the actual pressure gradient measured in mesenteric vessels (36) of corresponding branching orders as shown in Fig. 5, indicate a much steeper transition in the wing occurring between 2nd- and 3rd-order vessels.

In Fig. 6, the absolute pressure distribution as calculated from the MPG is compared with that predicted by an extensive mathematical model of the wing vasculature (20) and with that measured directly in consecutive branching orders (34). The three methods yield results in close agreement, with the pressure predicted by the model being slightly higher at most branching orders.

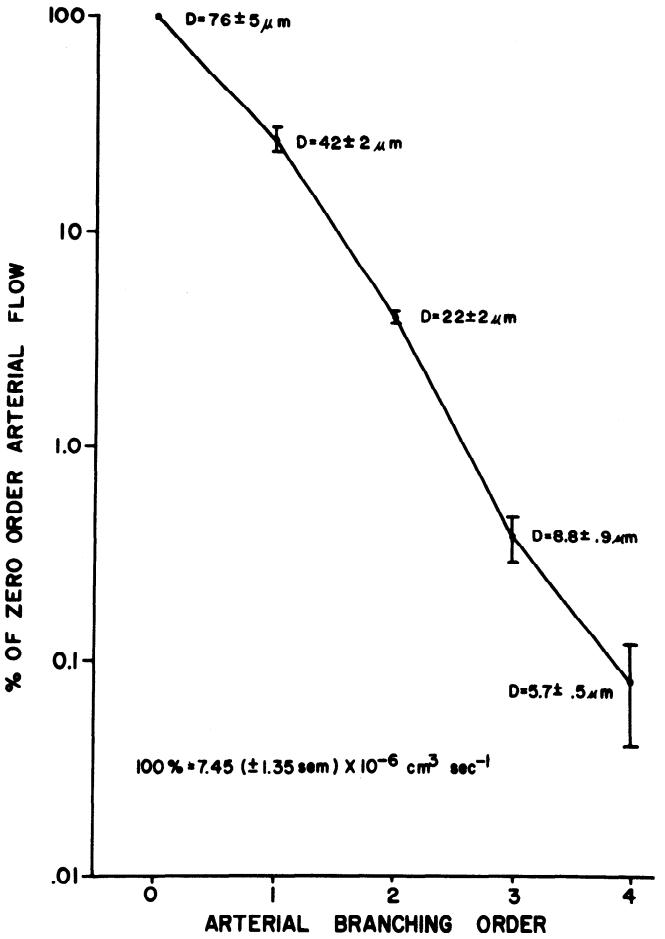

FIG. 3. Blood flow distribution expressed as a percentage of flow in supplying artery (0-order branch).

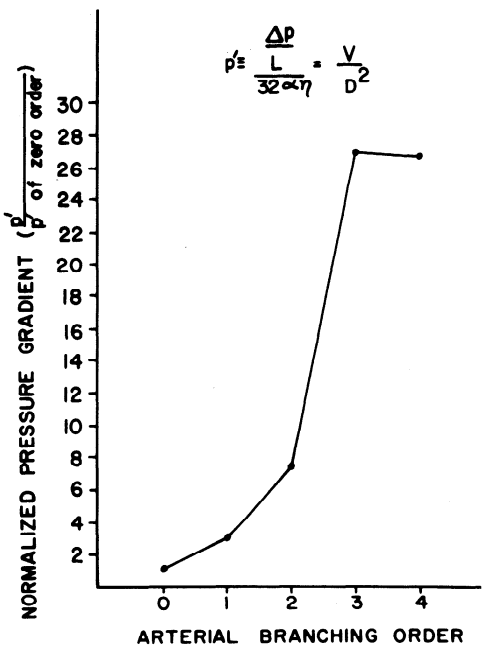

FIG. 4. Values of modified pressure gradient (p') computed from mean values of velocity (V) and (D) in Fig. 1 and expressed relative to 0-order branch value.

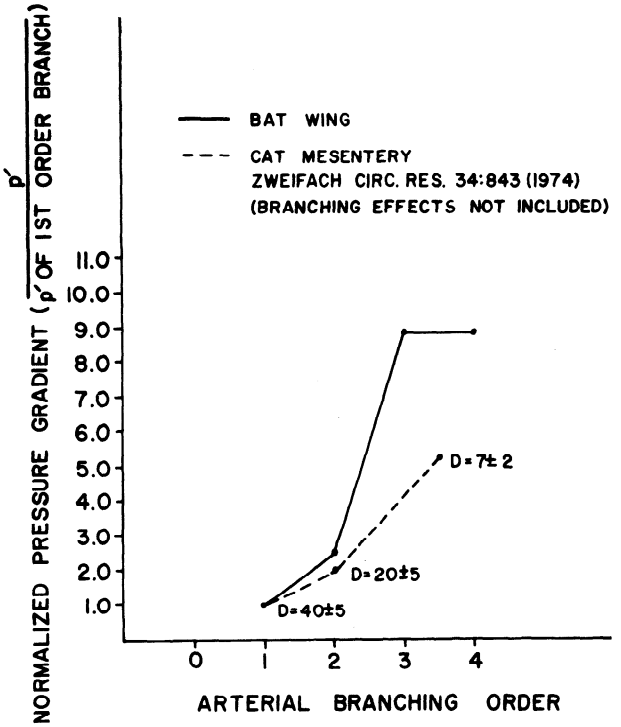

FIG. 5. Comparison between relative value of modified pressure gradient in bat wing to relative value of measured pressure gradient in cat mesentery (36). In each case relative values are computed as ratio of appropriate pressure gradient to the value in 1st-order vessel. D values are diameters for mesenteric preparation. Corresponding diameters in bat's wing are shown in Fig. 1.

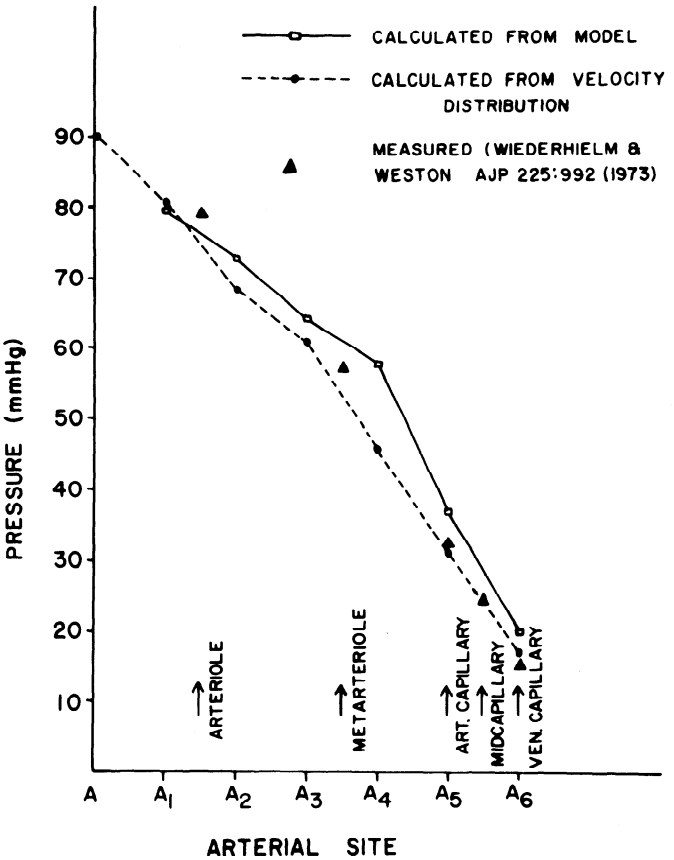

FIG. 6. Comparison of pressure distribution in bat wing as predicted from an extensive topological model (20), calculated from data of present study, and mean values of pressure directly measured at corresponding branching sites (34).

DISCUSSION

The distribution of velocities found in the present study of the bat wing shows a trend for lower velocities as the capillaries are approached. The value of about 0.5 mm s^{-1} measured in the capillaries is in close agreement with values reported for the minimally traumatized tenuissimus muscle (27), the cat mesentery (13), and the human nailfold (5). However, all velocities obtained are considerably lower than those reported for the rabbit omentum (9, 24). Whether this discrepancy is related to differences in anatomical arrangement of vessels, the function of the tissue under observation, the absence of anesthesia in the present study, or to possible traumatization effects is unknown. The effects of trauma incurred during tissue preparation have been shown to produce a large increase in measured blood velocity (28). Since in the present study trauma is minimal, it is possible that the lower values reflect a more nearly normal state than is possible with other experimental preparations.

Shear rates calculated from the measured velocities and diameters are found to be smallest in the 0-order vessels and to increase progressively down to 3rd-order vessels with a slight but nonsignificant decrease in 4th-order vessels. These findings are consistent with the basic trend reported in the cat mesentery for wall shear stress (16). Phenomenologically this result indicates that branching produces a greater reduction in vessel diameter than blood velocity. The resultant increase in wall shear rate may be part of the explanation for the greater difficulty in inducing adhering platelet aggregates in the smaller arterial vessels (33). Furthermore, in view of the fact that shear rate is an important determinant of whether circulating leucocytes will become adherent and remain adherent to vascular endothelium following the application of leucotactic stimuli (19), the present findings indicate that adherence would be less prominent in arterial branches which are more distal to the 0-order artery. This prediction is borne out by direct microscopic observation in our laboratory.

The blood flow distribution as calculated from the velocity and diameter distributions shows a logarithmic dependence on branching order. A similar dependence has been shown for the cat omentum (12) when flow was expressed as a function of vessel diameters, independent of position in the vascular tree. In the present study, the capillary volume flow is approximately 1.5 times higher than would be calculated on the basis of the average number of capillaries per distributing artery (30). A partial explanation for the difference is related to intermittent flow that normally occurs in terminal arterioles and capillaries. In the normal microcirculation not all capillaries have an active flow at the same time. In the present study the initial selection of capillaries for ve-

locity measurement was restricted to those with flow. Since the data reported herein are the velocities when flow was present, there is a tendency to overestimate the average flow since only the population of capillaries with flow are included. Based on the relative periods of terminal arteriole constriction as shown by Wiedeman (31), the ratio of capillary no-flow to flow time under normal conditions is approximately 0.25. Inclusion of this factor would tend to bring the measured and calculated values closer together.

The method of using the modified pressure gradient to estimate the pressure distribution yielded results in good agreement with those predicted from an extensive model of the wing vasculature (20) as well as with values reported experimentally (16). The largest difference occurs between the previous model prediction which shows a slightly larger pressure in most arterial vessels. The explanation for this lies in the fact that the average diameters used in the model differed from those used in the present study. The principle difference between the two sets of data was that the average diameter of the 1st-order vessel was 52 μm for the model compared to 42 μm for the average diameter of the 1st-order vessels in this study. The larger 1st-order diameter of the model would result in slightly higher predicted pressures than those actually measured. The close agreement between measured and calculated values suggests that, in spite of rheologic and topological complexities intrinsic to the vascular network, pressure distribution may be reasonably well predicted by contiguous measurements of center-line velocity and vessel diameter.

Furthermore, the lack of significant divergence between the calculated pressure distribution (using the MPG) and the measured pressure distribution obtained by Wiederhielm and Weston (34) implies that the product $\alpha\eta$ appearing in *equation 4* is a constant, independent of branching order. To have an $\alpha\eta$ product reasonably independent of branching requires tendencies for η to decrease with branching order to be offset by tendencies for α to increase. Since the present findings show an increase in shear rate with increasing branching order, a decreased viscosity would be anticipated (26). Because of the branching pattern and associated plasma skimming effects (2, 4, 23), a reduced local hematocrit with increasing branching order is also to be expected. Both of these factors – increased shear rate and reduced hematocrit – would result in a viscosity that decreased with branching order, though in view of the shear rates present, hematocrit changes are probably more significant (26). On the other hand, the quantity α which characterizes the relationship between the mean velocity and the center-line velocity would tend to increase from an ideal value of 0.5 for a parabolic velocity profile in the larger vessels to a value close to unity in the capillaries.

An estimate of the required change in hematocrit (H) to maintain a constant $\alpha\eta$ product may be made by employing the viscosity dependence on hematocrit relationship previously developed by Mayrovitz et al. (18). Using a and b to distinguish two different branching orders, the condition for equal products requires that

$$\alpha_a[1 + 2.5\ H_a/(1 - H_a)] = \alpha_b\ [1 + 2.5\ H_b/(1 - H_b)]$$

Assuming a 40% hematocrit in the supplying artery and α values of 0.5 and 1.0 in the artery and capillary leads to a required capillary hematocrit of 11.8%. Though not measured in the present study, this calculated capillary hematocrit value lies between the 9% value measured in the cat mesentery (13) and 17.2% average hematocrit measured in vessels 5–15 μm in the rabbit omentum (24) and is close to the 13% value measured in 8.7-μm glass tubes supplied by a 40% hematocrit reservoir (2). It is unknown whether the magnitude of the inverse tendencies described above is a unique property of the bat-wing vasculature, or if it has a more general application. Few, if any, other microvascular preparations provide the experimenter the opportunity to clearly establish the hierarchical position of the vessels as with the bat wing. Judgments of anatomical position based on vessel diameter alone can be misleading in view of the dynamic and often unpredictable nature of vasomotion.

In summary, the present study provides basic data on the variation in hemodynamic quantities in consecutive branching vessels from the supplying artery through the capillaries. Blood velocity is shown to decrease and shear rate is found to increase as the capillary is approached. It is suggested that the increase in shear rate may be a factor that renders platelet aggregation and leucocyte sticking less prominent in arterial branches closer to the capillary bed. Furthermore, the employment of the modified pressure gradient concept was shown to be a reliable and useful method to obtain a measure of the distribution of pressure within consecutive vascular segments. Based on the analysis of the data, it is concluded that there is a continuous decrease in effective blood viscosity as the capillary bed is approached. This decreased viscosity is mainly due to a reduction in local hematocrit and may be reasonably well predicted when changes in velocity profile are taken into account.

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