The Influence of Erythrocyte Concentration upon the Pressure-Flow Relationships in the Dog’s Hind Limb

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Since alterations in cell-plasma ratios are of paramount importance in the assessment of peripheral resistance, a study of pressure-flow relations was made in which blood having wide hematocrit values was perfused through a dog’s hind limb in which collateral circulation has been excluded. When vessels were moderately dilated by denervation, the relative apparent viscosity was not affected by the perfusion pressure. When, however, the vessels were maximally dilated after a preceding period of anoxia, the relative apparent viscosity was found to become greater as perfusion pressure was progressively reduced. The in vivo relative apparent viscosity did not significantly exceed that of plasma until the erythrocyte concentration reached 30 per cent.

A very marked difference has been observed between the apparent viscosity of blood measured in living tissues and the values obtained in capillary viscometers. The most comprehensive study of the relationships between the erythrocyte concentration of the blood and its apparent viscosity in living tissues was reported in 1933 by Whittaker and Winton. However, several cardinal features of this work were subjected to criticism by Green and his collaborators. Two of the principal objections were that the blood vessels in the isolated perfused extremities were maximally dilated, and that no precautions were instituted to prevent outflow of blood from nonligated collateral channels.

Since it is of paramount importance to be able to gauge the change in peripheral resistance incident to any alteration in hematocrit ratio under a wide variety of physiologic and pathologic conditions, this problem was reinvestigated using improved experimental technics.

METHODS

In dogs anesthetized with morphine and sodium barbital (200 mg. per kilogram), the relationships between pressure (P) and flow (F) were established for the hind limb over a wide range of pressures. Studies were performed under essentially two degrees of vasodilatation in different experiments. In one series, the vessels were maximally dilated by means of a preceding period of ischemia, and the hind limb was perfused with anoxicemic blood from a glass reservoir. In the other series, the vascular bed was dilated to a lesser extent by transection of the sciatic and femoral nerves, and was perfused directly from the animal through the femoral artery.

1. Denervated Preparation

In 15 experiments, the nerves to the leg were sectioned prior to any measurements of flow in order to preclude neurogenic alterations of vasomotor tone throughout the course of the experiment. The femoral artery and vein were dissected free, and after the administration of heparin, T-cannulas were inserted into both vessels. Collateral circulation was prevented by applying two wire tourniquets about the remaining tissues of the upper thigh by means of specially constructed érasureurs, as described by Green and colleagues.

Venous outflow was determined by temporarily occluding the femoral vein cephalad to the T-cannula, and collecting the outflow from the sidearm in a vertical glass tube of constant internal diameter. The changing level of hydrostatic pressure in this vertical cylinder was registered optically by means of a Gregg manometer, as previously described. The slope of the record so obtained was converted to flow by means of repeated calibrations with blood obtained during each flow estimation. The height of the outflow tube was periodically adjusted to the zero level of the prevailing femoral venous pressure.

Mean femoral arterial pressure was registered by a damped Gregg manometer via the T-cannula in the femoral artery. Variations in pressure were produced by means of a screw-clamp placed about the
femoral artery centrally to the T-cannula. Perfusion pressure was computed as the difference between arterial and venous pressures. Limb temperatures were read at frequent intervals from a thermometer inserted deeply into the lower thigh muscles.

After a pair of control P:F curves were obtained as previously described, the erythrocyte concentration was altered by replacing an arbitrary quantity of the experimental animal's blood with either plasma or packed erythrocytes. Five such variations in hematocrit ratio were produced in most experiments, and the P:F relationships were determined after each change, allowing a minimum of 20 minutes for mixing and stabilization. At the middle and end of each experiment, volumes of donor plasma or erythrocytes estimated to return the hematocrit values as close as possible to the control level were infused, in order to detect any fortuitous alterations of peripheral resistance. Donor blood was obtained during the same day as the experiment in practically every case, and refrigerated blood more than one day old was never used.

2. Maximum Vasodilatation

In this series of nine experiments, the hind limbs were prepared in the same manner, including denervation and the insertion of T-cannulas into the femoral artery and vein. Venous outflow was measured as before, but the outflow tube was kept at the hydrostatic level of the femoral vein, so that venous pressure in this series was zero. In order to produce maximum vasodilatation, the hind limb was rendered ischemic for a minimum of 10 minutes by occluding the femoral artery proximal to the T-cannula. During this interval, the oxygen tension of the donor blood was considerably lowered by causing it to perfuse the hind limb at least three complete circulations prior to P:F determinations. In six of these experiments, the maximal degree of dilatation was further ensured by the addition of 20 mg. of sodium nitrite to every 100 cc. of donor blood. This high concentration of nitrite, aside from its vasodilating action, visibly oxidized the hemoglobin to methemoglobin, thereby further lowering the oxygen content of the donor blood. It was determined viscometrically that this concentration of nitrite did not alter the rheologic properties of the blood significantly.

For the actual P:F determinations, the heparinized donor blood was caused toperfuse the extremity from a glass reservoir immersed in a constant temperature water bath maintained at 37 C. Five stages of air pressure (from 30 to 120 mm. Hg) were applied alternately to the surface of the blood in this reservoir by opening the stopcocks above the appropriate mercury-column valves, and this was usually repeated a second time to obtain a total of 10 points for construction of the actual P:F curve at each hematocrit value. The perfusion reservoir was connected to the vertical portion of the T-cannula in the femoral artery, and perfusion pressure was optically recorded. Constant perfusion pressures were achieved by interposing a large air chamber between the mercury-column valves and the blood reservoir. The blood was well mixed by gentle swirling prior to P:F determinations, and by the turbulence created by the frequent return of portions of this blood after each individual measurement. After an entire P:F curve was constructed at a given hematocrit ratio, the clamp was removed from the femoral artery, and the limb circulation was restored through regular channels for a period of approximately 45 minutes before any subsequent P:F measurements were conducted. In addition, the P:F curves for plasma were recorded at the beginning and end of each experiment to ascertain whether any significant alterations in resistance had developed.

3. Viscosity Determinations in Vitro

In all experiments, the viscosity of each erythrocyte concentration was measured by means of a high velocity viscometer, as recommended by Whittaker and Winton. In approximately two-thirds of our experiments, the capillary tube used had a constant internal diameter of 0.876 mm., and was 254.5 mm. in length. The viscometer tube used in the remaining experiments had a diameter of 1.048 mm., and a length of 254.2 mm. The viscosities of all samples were measured at pressures of 100, 75, and 50 mm. Hg. The hematocrit readings were determined by centrifugation in Wintrobe tubes at 3000 R.P.M. for 30 minutes, at a mean radius of rotation of 160 mm. No corrections were made for plasma trapping.

In approximately half the experiments, plasma protein concentrations were determined photocolorimetrically by the biuret reaction. No significant alterations in the level of plasma proteins were observed in the course of any of these experiments.

Results

1. Pressure-Flow Relationships

(a) Denervated Preparation. In all experiments, a distinctly curvilinear relationship was observed between pressure and flow. These curves were invariably convex to the P axis, and at least 90 per cent of these curves described a straight line when plotted on log-log coordinates as practiced by Green and co-workers. Figure 1 illustrates an experiment which is typical of this group. The lines...
representing the relationships between P and F at each hematocrit ratio were obtained by the method of least squares applied to the empirical equation \( F = c P^n \), where \( c \) and \( n \) are constants. The exponent \( n \) is the slope of the logarithmic P:F curve, and the coefficient \( c \) is equal to the flow at \( P = 1 \text{ mm. Hg} \). Such a curve, if extrapolated, would intersect the origin of the coordinate system.

In figure 1, the line signifying a hematocrit value of 43.2 per cent (curve 1) represents the control relationship between P and F when the hind limb was perfused with the experimental animal's unaltered blood. When donor plasma was substituted for an equivalent quantity of the recipient's blood, the erythrocyte concentration was reduced to 26.4 per cent, and the curve is shifted significantly to the left (curve 2). Subsequent replacement of packed donor corpuscles for an equivalent quantity of the experimental animal's cell-poor blood augmented the hematocrit ratio to 55.8 per cent, which is above the control value. This is depicted by curve 3, which lies to the right of the control curve 1. Subsequent exchanges of packed red blood corpuscles for whole blood yielded hematocrit values of 68.4 and 79.8 per cent; these are delineated by curves 4 and 5, respectively. It is quite evident from the figure that an augmented corpuscular concentration results in a markedly increased resistance to blood flow. This is illustrated most dramatically by the considerable displacement of curve 5 downward and to the right. When some of this polycythemic blood was exchanged for plasma, however, the curve immediately reverted toward normal values (curve 6). In this experiment, the hematocrit ratio returned to 55.1 per cent, virtually identical with the third value of 55.8 per cent. It is obvious that curve 6 is practically coincident with curve 3, within the limits of experimental error. Therefore, we may conclude that no significant alterations in vasomotor tone were manifest in this particular experiment.

It should be noted at this point that all curves plotted in figure 1 are virtually parallel to each other. All values for the exponent \( n \) in this experiment ranged between 1.3 and 1.6, as determined by the method of least squares.

In the experiment showing the greatest degree of parallelism, all values of \( n \) varied between 1.37 and 1.45 for a range of hematocrit ratios between 30.1 and 59.6 per cent. In the entire series of experiments, although some random variations were noted, there was never any regularly observed change in slope coincident to variations in erythrocyte concentration.
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the direction of the ordinate, or F axis. A curvilinear plot convex to the P axis was never observed either for plasma or blood under conditions of maximum dilatation.

Figure 2 illustrates a typical experiment of this group. The initial P:F curve (curve 1) was derived by perfusing the ischemic hind limb with plasma containing 2.5 per cent red blood corpuscles. This contamination with erythrocytes resulted from the fact that preliminary irrigation of the limb with 100 cc. plasma was insufficient to eliminate all residual blood. The limb was perfused at 11 different pressures over a range between 18 and 128 mm. Hg, and it is evident that the relationship is virtually linear until flows exceed 600 cc. per minute, when the curve splays to the right. When the limb was subsequently perfused with blood of increasingly higher erythrocyte concentrations, it is clear from figure 2 that the curves are shifted further and further to the right, and that the slopes progressively diminish. A slight convexity to the F axis is evident at the upper ends of curves 2 and 3 (representing hematocrit ratios of 30.8 and 47.9 per cent, respectively), while the P:F values for curves 4 and 5 (58.4 and 73.8 per cent, respectively) lie along a perfectly straight line. When the hind limb was finally perfused again with plasma (contaminated with 5.4 per cent erythrocytes), the P:F relationships were almost identical with the original values for plasma. The figure reveals that curve 6 is virtually collinear with curve 1, within the limits of the experimental methods, indicating no significant alterations in the vascular resistance of the limb in the 4.5 hour period between plasma perfusions.

By the method of least squares, the linear portions of all these curves were fitted to the general equation \( F = a P + b \), where \( a \) is the slope and \( b \) the F axis intercept. The equations so obtained were employed in drawing the linear portions of all curves included in figure 2. The slope decreased progressively from a value of 7.7 for plasma (curve 1) to a value of 4.0 for blood with a hematocrit reading of 73.8 per cent (curve 5). Also, the extrapolated values for P at F = 0 were computed for each curve. Figure 2 reveals that the P axis intercept progressively increases the greater the erythrocyte concentration of the blood. At F = 0, the P intercepts are 13 and 5 mm. Hg for the first and last plasma perfusions (curves 1 and 6), while the intercept is 29 mm. Hg for a hematocrit ratio of 73.8 per cent (curve 5). This concordant relationship between erythrocyte concentration and P axis intercept confirms the previous findings of Whittaker and Winton. However, in view of Burton's concept of the "critical closing pressure," it is debatable whether we are justified in extrapolating these curves to F = 0 without obtaining more points at the lower end of the flow scale.

2. Relative Apparent Viscosity Determinations

(a) Denervated Preparation. For the determination of apparent viscosity in vivo, only those experiments where flow changed less than 20 per cent at comparable hematocrit ratios during a given experiment were subjected to a detailed analysis. By this criterion,
only 6 out of 15 denervated preparations were considered to be sufficiently free from fortuitous alterations of vasomotor tone. Also, since the erythrocyte concentration of the entire blood volume could not be made to approach zero without first producing reactive hyperemia and then threatening the life of the animal, the lowest hematocrit value reached in any experiment was 22 per cent. The apparent viscosity of plasma then had to be deduced by extrapolation, which of course introduces an additional error.

It has already been shown that the curves of log P against log F are virtually parallel to each other over a wide range of hematocrit values (figure 1). This has very important implications as far as relative apparent viscosity is concerned. If the equations

\[ F_1 = c_1 P^{n_1}, \quad \text{and} \quad F_2 = c_2 P^{n_2} \]

represent the relationships between P and F for blood of any two hematocrit values, then the proportionality

\[ \frac{F_1}{F_2} = \frac{c_1 P^{n_1}}{c_2 P^{n_2}} \]

represents the ratio of the flows of these two samples at any given pressure. If the P:F curves for these two samples are parallel when plotted logarithmically, then \( n_1 = n_2 \), and \( F_1/F_2 = c_1/c_2 \). It follows, therefore, that the relative flows are completely independent of pressure. If \( F_1 \) is taken as the flow of plasma and \( F_2 \) that of blood of any given corpuscular concentration, then the relative apparent viscosity

\[ \frac{\eta_2}{\eta_p} = \frac{F_1}{F_2} = \frac{c_1}{c_2} = K \]

is also constant for any given sample of blood over a wide range of pressures when the vascular bed is not maximally dilated.

In practice, the relative apparent viscosities were computed by arbitrarily comparing flows at a pressure of 100 mm Hg. The value of F was plotted against the hematocrit ratio for each experiment, and the flow at a 45 per cent corpuscular concentration was estimated by interpolation. The composite graph for all suitable experiments in this series is reproduced in figure 3, which gives an indication of the range of variations between individual experiments. This curve was then extrapolated to zero hematocrit ratio, and the apparent viscosities for the various erythrocyte concentrations were computed against this extrapolated value for plasma to yield the middle curve of figure 4. This figure shows that the relative apparent viscosity increases progressively with corpuscular concentration, and that the rate of change increases markedly at the higher levels of hematocrit ratios. In
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addition, the figure reveals that relative apparent viscosity is consistently greater in the denervated preparation than in the maximally dilated limb (computed at P = 100 mm. Hg), and that this effect is increasingly exaggerated as corpuscular concentration rises.

(b) Maximum Vasodilatation. In this series of experiments, the apparent viscosity of blood flowing through the ischemic hind limb was readily determined relative to plasma simply by comparing the flows of blood and plasma at equivalent pressures. Using the same criterion for vasomotor stability as stated above, only five of the nine experiments in this series were deemed suitable for analysis.

From the forms of the P:F curves for plasma and blood under conditions of maximal dilatation (fig. 2), however, it is not immediately evident whether the relative apparent viscosity is dependent upon the reference pressure chosen. If \( F_p = a P + b \) and \( F_b = c P + d \), where \( F_p \) and \( F_b \) are the flows of plasma and blood, respectively, then the relative apparent viscosity of this sample of blood will be given by the proportionality

\[
\frac{\eta_b^*}{\eta_p^*} = \frac{F_p}{F_b} = \frac{aP + b}{cP + d}
\]

where \( \eta_b^* \) and \( \eta_p^* \) are the apparent viscosities of blood and plasma, respectively. Since the relationships between the constants \( a, b, c, \) and \( d \) are not known, the influence of reference pressure must be ascertained from the experimental data.

The apparent viscosities relative to plasma for the bloods of varying corpuscular concentration were computed from the data in figure 2 at \( P = 40, 70, \) and 100 mm. Hg, respectively, using the least squares equations. The results are plotted in figure 5. It is evident from this figure that for any given hematocrit ratio, the relative apparent viscosity increases as the pressure declines. Also, the effect is exaggerated at higher erythrocyte concentrations.

Thus, in the maximally dilated limb, the relative apparent viscosity is very significantly dependent upon the level of pressure involved. For the sake of comparison with the relative apparent viscosity in the denervated preparation and in the glass viscometer tube, the viscosities in the five suitable experiments of this series were determined arbitrarily at a pressure of 100 mm. Hg, and the average results appear in the lowest curve of figure 4. It is evident that the apparent viscosity of blood is only slightly greater than plasma up to a hematocrit ratio of about 30 per cent. The slope of the curve progressively increases, however, so that at an erythrocyte concentration of 80 per cent the apparent viscosity of blood is 2.8 times that of plasma, in the maximally dilated limb. These values are close to, but somewhat lower than, those reported by Whittaker and Winton,\(^1\) when their values are computed relative to plasma rather than to water.

(c) Comparison of Viscosity in Vitro and in Vivo. The viscosities of all blood samples were compared with the viscosity of plasma from the same experiment by means of a high-velocity viscometer. The average results are depicted in the upper curve of figure 4. The latter demonstrates that the relative apparent viscosity of blood is considerably greater when measured in a viscometer as compared with the values obtained by perfusing a hind limb, thus confirming the previous findings of Whittaker and Winton.\(^1\)

The in vitro viscosity measurements served as a valuable control in our experiments. Any agglutination of erythrocytes due to incompatible blood reactions or to sludging would...
have been indicated by an abnormally high apparent viscosity value. Furthermore, the finding that the addition of sodium nitrite to blood did not alter the relationship between apparent viscosity and hematocrit ratio justified its use in some of our perfusion experiments. Other studies have indicated that sodium nitrite does increase the viscosity of blood, but a parallel change in erythrocyte volume occurred concomitantly; hence, the relationship between viscosity and corpuscular concentration was not appreciably altered.

**DISCUSSION**

A considerable difference of opinion exists in the literature concerning the nature of the relationship between flow and perfusion pressure. Whittaker and Winton described a rectilinear plot of P against F for the isolated hind limb of the dog, and these findings have been confirmed in the hind limb of the rabbit by Nichol and his collaborators. Pappenheimer and Maes also felt that the P:F relationship was linear over a wide range, but detected a deviation from linearity below critical values of F. On the other hand, Green and his co-workers reported that F was an exponential function of P, and this was subsequently confirmed in our laboratory.

Green suggested that the linearity between P and F observed in the experiments of Whittaker and Winton could be the result of one or several of the following factors: (a) failure to ligate the cut ends of collateral vessels, (b) lapse of two to three hours prior to making experimental observations, (c) denervation of the limb, (d) limited range of pressures employed, and (e) extreme dilatation of the vascular bed. The present studies make it clear that (e) is the only valid explanation for the divergent results. In our studies, collateral circulation was adequately controlled and experimental observations were made just 10 minutes after interrupting the normal circulation to the limb, and yet P:F curves were still linear when the vascular bed was maximally dilated. Even when a wider range of pressures were employed, a linear P:F curve was still obtained, or at very high flows the curve splayed in a direction convex to the flow axis. Finally, it has been shown that denervation of the limb, per se, does not produce a linear P:F relationship, since parabolic curves resulted when the vascular bed of the denervated limb was not maximally dilated.

By a process of elimination, therefore, linearity between P and F appears to be dependent upon the maximum dilatation of the blood vessels. Green explains the exponential relationship under more physiologic conditions on the basis of a passive increase in the diameter of arterioles or an opening of previously dormant capillary beds incident to an increase in perfusion pressure. With maximum dilatation, no new capillary beds can be mobilized. Furthermore, the distensibility of the arteriolar wall is limited by a basketwork of connective tissue fibers surrounding each arteriole. Alexander has shown that the vascular smooth muscle exhibits a considerably lower modulus of elasticity than does the fibrous tissue, and that the fibrous tissue is dominant at the upper end of the pressure-volume diagrams. He has also demonstrated that the greater the degree of vasoconstriction (produced by epinephrine), the more important is the role played by the relatively distensible muscular elements.

This helps explain the observations reported by Green and co-workers that the logarithmic slope, n, increases with vasoconstriction, but approaches unity with vasodilatation. The greater the value of n, the more pronounced would be the reduction in computed resistance for a given rise in pressure. Such a finding would be consistent with the predominant activity of the distensible smooth muscle during constriction. Conversely, as n approaches one, the vascular bed behaves more like a system of rigid tubes. This could be ascribed to the limiting network of connective tissue fibers which assume prominence during vasodilatation.

Although most of the curves relating P to F were perfectly linear under conditions of maximum vasodilatation, a deviation which was convex to the F axis was not infrequently seen at very high flows (fig. 2). We believe that this phenomenon is dependent upon turbulence resulting from the high velocity of flow.
In the case of homogeneous fluids, turbulence does not develop unless the Reynold's number is significantly greater than 1000. Coulter and Pappenheimer\textsuperscript{12} have reported a lower critical Reynold's number ($R_c$) averaging 970 in the case of blood in vitro, and Müller\textsuperscript{13} has reported a gradual deviation from laminar flow in the case of heterogeneous fluids beginning at $R_c = 700$. Observing the velocity profile of injected dye by means of high speed cinematography, McDonald\textsuperscript{14} has stated that the $R_c$ is about 300, and certainly not greater than 550, in the case of pulsatile flow within the large vessels. When calculated on the basis of $R_c = 1000$, the flow of plasma would be turbulent in all tubes of internal diameter of 4 mm. or greater when flow exceeds 600 cc. per minute. If calculated on the basis of the lower $R_c$ values cited above, turbulence would develop in considerably smaller channels. Since the rubber tubing used in perfusing the limb and in measuring flow had an internal diameter greater than 4 mm. in our experiments, this bend in the linear graphs at high flows could, at least partially, be an artefact.

With respect to the apparent viscosity of blood, its anomalous nature has been amply established by numerous carefully performed studies which have recently been exhaustively reviewed by Bayliss.\textsuperscript{16} The principal characteristics which distinguish heterogeneous fluids rheologically are the variations in apparent viscosity which are dependent upon the internal diameter of the tube and upon the mean velocity of flow. It has been demonstrated unequivocally that the apparent viscosity diminishes as the radius of the tube decreases,\textsuperscript{13, 15, 16} while the apparent viscosity increases when mean velocity falls below a certain critical level.\textsuperscript{13, 17, 18}

Whittaker and Winton\textsuperscript{1} were the first to observe that relative apparent viscosity is markedly less when measured in a perfused tissue than in a high-velocity viscometer tube. They ascribed this difference to the effect of the small diameter of the arterioles and capillaries upon the anomalous rheologic properties of blood, and to the lowering of erythrocyte concentration in the smaller vessels.\textsuperscript{19} They also observed that the relative apparent viscosity increased at lower pressures, and to this they imputed the anomalous effects of diminished velocity of flow. Our data directly confirm the reduced apparent viscosity of blood in perfused tissues as compared with a glass viscometer tube (fig. 4), and the decrease in apparent viscosity at higher pressures in the maximally dilated limb (fig. 5). However, we are unable to account for the absence of a significant alteration in relative apparent viscosity over a wide range of pressures when the vascular bed is not maximally dilated.

**SUMMARY**

The relationships between pressure and flow were determined in the hind limb of the dog over a wide range of hematocrit values under conditions of moderate vasodilatation (denervated preparation) and of maximum dilatation (perfusion with anoxemic blood after 10 minutes or more of ischemia). In the denervated preparation, the P:F curves were parabolic, and convex to the pressure axis. The logarithmic slopes were equal for all hematocrit ratios in any given experiment. Therefore, the relative apparent viscosities were independent of variations in pressure.

When dilatation was maximal, the curves relating pressure and flow were virtually linear, with occasional deviations concave to the pressure axis at very high flows. In these experiments, the relative apparent viscosity was intimately dependent upon pressure, becoming significantly greater when progressively lower reference pressures were chosen.

The apparent viscosity of blood as measured in the tissues of the hind limb was only slightly greater than that of plasma until the erythrocyte concentration exceeded 30 per cent. Above this value, the relative apparent viscosity became significantly elevated as the upper limits of hematocrit ratio were approached. The relative apparent viscosity as measured in vivo was markedly less than the values obtained in a high-velocity viscometer.

**REFERENCES**

\textsuperscript{1} WHITTAKER, S. R. F., AND WINTON, F. R.: The apparent viscosity of blood flowing in the
isolated hindlimb of the dog, and its variation with corpulmonary concentration. J. Physiol. 78: 339, 1933.


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