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Rheological effects of red blood cell aggregation in the venous network: A review of recent studies

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Abstract. It has long been recognized that understanding the rheological properties of blood is essential to a full understanding of the function of the circulatory system. Given the difficulty of obtaining carefully controlled measurements *in vivo*, most of our current concepts of the flow behavior of blood *in vivo* are based on its properties *in vitro*. Studies of blood rheology in rotational and tube viscometers have defined the basic properties of blood and pointed to certain features that may be especially significant for understanding *in vivo* function. At the same time, differences between *in vivo* and *in vitro* systems combined with the complex rheological properties of blood make it difficult to predict *in vivo* blood rheology from *in vitro* studies. We have investigated certain flow properties of blood *in vivo*, using the venular network of skeletal muscle as our model system. In the presence of red blood cell aggregation, venous velocity profiles become blunted from the parabolic as in Poiseuille flow, as pseudo-shear rate (= mean fluid velocity/vessel diameter) is decreased from $\sim$100 s^{-1} to 5 s^{-1}. At control flow rates, the short distance between venular junctions does not appear to permit significant axial migration and red cell depletion of the peripheral fluid layer before additional red cells and aggregates are infused from a feeding tributary. Formation of a cell-free plasma layer at the vessel wall and sedimentation *in vivo* are evident only at very low pseudo-shear rates (< 5 s^{-1}). These findings may explain in large part observations in whole organs of increased venous resistance with reduction of blood flow.

Keywords: Red blood cell sedimentation, axial migration, velocity profiles, venous vascular resistance, blood viscosity

1. Introduction

The aggregating property of red blood cells was first described by John Hunter in 1786 and was long considered to be principally of pathophysiologic importance since aggregation is elevated in many disease states; hence the term “blood sludging” coined by Knisely [25] to describe the phenomenon. It has been suggested that under low flow or circulatory shock conditions red cell aggregates would significantly impede the flow of blood *in vivo*. However, red cell aggregation is also normally present in humans and in many other “athletic” species while it is absent in sedentary animals [32]. This raises the possibility that normal levels of aggregation may serve a homeostatic function. In this report we describe evidence from recent studies that red cell aggregation provides a means to automatically adjust venous vascular resistance in skeletal muscle in accordance with the blood flow through the muscle.

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2. Effect of aggregation on blood flow *in vivo*

Suggestions that red cell aggregation may have a significant effect on circulatory function have stimulated a number of studies of the peripheral circulation under normal and low flow conditions. Studies by Gustavsson et al. [22] of the relationship between the perfusion pressure and blood flow through muscles of the dog hind limb found only a very small, perhaps non-significant, increase in vascular resistance with increased red blood cell aggregation. However, to minimize effects of active changes in vessel diameter in those studies the vascular bed was maximally dilated. Consequently, at normal arterial pressure the flow and shear rates were much higher than normal while at the lowest arterial pressure studied the flow rate corresponded to that seen at normal arterial pressure. Thus the effects of red cell aggregation may have been masked over most of the range of arterial pressure. Microcirculatory studies under conditions of normal vascular tone in several laboratories [26,31,37] have shown that elevating red cell aggregation can reduce blood flow in microcirculatory vessels to varying degrees, and it has been suggested that this may be due to blockage of precapillary vessels [26].

Studies of venous vascular resistance have provided evidence that red blood cell aggregation has a very significant effect on *in vivo* hemodynamics. Pressure-flow studies on the muscles of the dog hind limb by Thulesius and Johnson [36] showed that lowering arterial pressure, which also reduced blood flow, caused a doubling of venous resistance when perfused with normal dog blood. This rise in resistance was diminished with hemodilution and it was suggested that red cell aggregation at low flow might be responsible for the rise in resistance. In more extensive studies using the cat lateral gastrocnemius preparation described by Mellander and coworkers [29], we found [10] that venous resistance fell 4 fold with a 20 fold increase in flow as shown in Fig. 1. To determine whether red cell aggregation was involved, the muscle was perfused with cat red cells suspended in 6% dextran 40 in Ringers solution, in which the cells do not aggregate. Under these conditions, venous resistance decreased by about 60% from that of normal blood at control flow rates in resting muscle (5 ml/min/100 g tissue) and there was only a slight, not-significant trend for resistance to decrease as flow was increased as shown in Fig. 1. Based on these considerations we suggested that normal red blood cell aggregation is responsible for about 60% of venous vascular resistance in resting muscle and is responsible for the variations in venous resistance as flow is altered. Since venous resistance represents normally only about 10% of total vascular resistance, such changes might not be evident when total vascular resistance was measured as in the studies of Gustavsson [22] cited above.

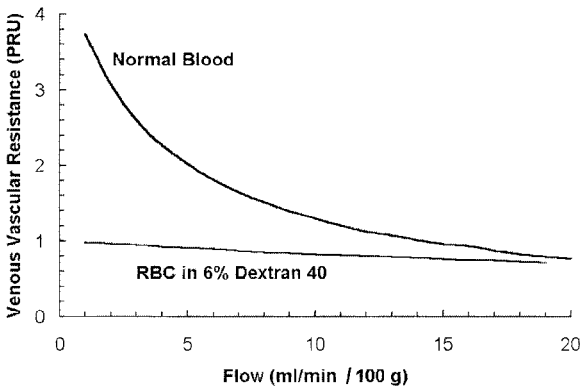

Fig. 1. Relation between venous vascular resistance and blood flow in the isolated cat lateral gastrocnemius muscle with normal blood and with red cells in 6% dextran 40 in Ringers solution. Data from Cabel et al. [11].

This finding suggests that red blood cell aggregation may have functional significance for normal physiology. A flow dependent change in venous resistance as seen in these studies would tend to make capillary pressure independent of blood flow [10,18,38]. This feature would be particularly important to maintain a normal fluid balance as well as maximizing blood flow in tissues such as skeletal muscle which undergo large changes in blood flow. It is also important to note in this regard that red blood cell aggregation is normally present in "athletic" species but not in sedentary species and is most pronounced in those species having the highest capacities for oxygen consumption as shown by Popel et al. [32]. Flow dependence of red blood cell aggregation in the venous vessels may represent a unique mechanism for circulatory regulation inasmuch as it utilizes the rheological properties of the blood and not the active responses of the vasculature. To understand how this mechanism might operate in the venous vascular network it is necessary to examine red blood cell aggregation and its effect on the flow properties of blood.

3. Mechanism of red cell aggregation

Red cell aggregates may form when the cells come into close proximity. One explanation often advanced for aggregation is the bridging hypothesis proposed by Chien and Jan [13] which postulates that long-chain macromolecules such as fibrinogen or dextrans of high molecular weight may be adsorbed onto the surface of more than one cell, leading to a bridging effect between cells. It has been proposed by other investigators that the reduced concentration of macromolecules in the vicinity of red cells lowers the osmotic forces in the vicinity, causing fluid to move away and increasing the tendency for adjacent red cells to come together [4,27,28]. According to both the bridging and the depletion theories, the total adherent force between two cells is maximal when the cells are oriented *en face*, thus it is not uncommon to observe cells arranged in *rouleaux.* The shear stress required to separate two cells in this orientation suspended in 4% dextran 70 was determined by Chien and coworkers to be less than 1 dyn/cm^2 [14]. The exact configuration of a group of cells depends on local conditions such as shear rate and cell concentration (hematocrit) and a variety of complex forms ranging from single rouleaux to a branching network of rouleaux to more compact spheroids may be seen.

4. *In vitro* studies of blood rheology

4.1. Blood rheology in rotational viscometers

Much of our current understanding of the rheological effects of red cell aggregation is based on studies in rotational viscometers by Chien and colleagues [11,12] as well as other investigators such as Brooks et al. [9], and Merrill and coworkers [30]. In these studies a thin film of blood is sheared between two concentric cylinders, creating a constant shear rate across the blood layer. The experiments are designed to obtain viscosity measurements when red blood cells and/or aggregates are uniformly distributed within the fluid layer. These values are shown in Fig. 2. Most of the increase at low shear rates is due to red cell aggregation since when red cells are suspended in a non-aggregating medium the change in viscosity is much less as also shown in Fig. 2. The effect of aggregation on apparent viscosity is due to the increase in effective particle size and greater trapping of the plasma [12]. As shear rate gradually decreases, the lower shear forces acting on the red cells allow larger aggregates to form, increasing effective viscosity.

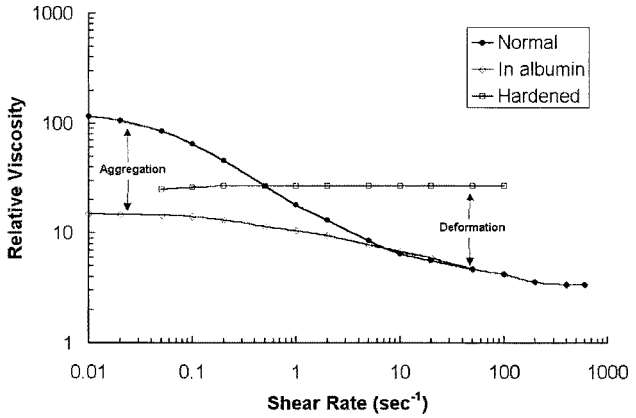

Fig. 2. Relation between shear rate and blood viscosity relative to plasma for normal human blood, red cells in albumin and red cells hardened by glutaraldehyde. The difference between the curves for normal blood and red cells in albumin represents the effect of red cell aggregation. Data from Chien et al. [12].

The residual effect of shear rate on viscosity in the absence of aggregation is due to the flexibility of the red blood cell. At very low shear rates, the orientation of the red blood cell relative to the direction of flow is random, but with increasing shear rate the flexibility of the red cell allows it to orient in a manner that presents a minimal cross-section to the flow stream, effectively reducing particle size. If the red blood cell is hardened by incubation in glutaraldehyde, the viscosity becomes almost independent of shear rate.

Comparison of Figs 1 and 2 reveals similar changes in viscosity and venous vascular resistance as a function of shear rate or flow rate in the presence and absence of aggregation. A minor difference is that venous resistance does not change significantly with flow in the absence of aggregation. These observations would appear to support the suggestion that the increase in venous vascular resistance with flow reduction is due to the formation of red cell aggregates. However, direct comparison of the two findings is limited by the fact that the systems in which the studies were carried out are quite different.

It is known that the change in venous vascular resistance in skeletal muscle with reduction in flow occurs in venules larger than 25 μm [24]. If red blood cell aggregation is indeed responsible for the changes in venous resistance with normal blood shown in Fig. 1, one would expect to find similar changes in viscosity in glass tubes of this diameter and greater as flow rate is altered.

4.2. Studies of blood rheology in tubes

Studies on viscosity of human blood in small glass tubes by Reinke et al. [33], as presented in Fig. 3 showed, unexpectedly, that apparent viscosity was essentially unaltered by changes in blood flow in the range seen in skeletal muscle venules of the cat [23]. Data obtained in tubes ranging from 31 to 94 μm diameter with a variety of feed hematocrits showed similar results. To understand why blood viscosity is independent of flow rate in small tubes it is necessary to consider the conditions that prevail *in vitro.*

Unlike the flow in rotational viscometers, in tube flow there is a gradient in shear rate which, together with the presence of the vessel wall, can lead to an asymmetry of forces acting on a particle in a suspension. In the case of red cells this leads to a radial motion in the direction of higher velocity, a property known as axial migration. Such migration leads to the formation of a cell-poor or even a cell-free plasma layer near the vessel or tube wall. The circumstances leading to the formation of such a layer can

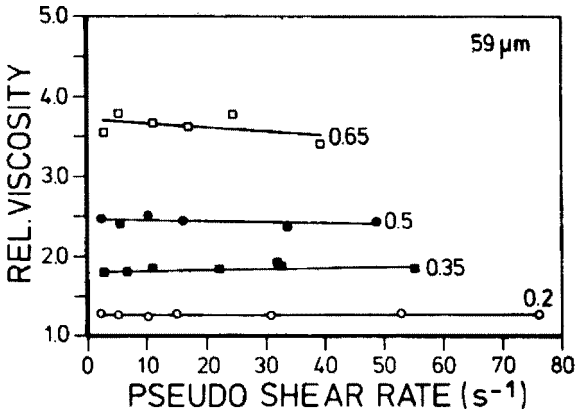

Fig. 3. Relation between flow (pseudo-shear rate) and relative apparent blood viscosity for human blood in a tube of 59 μm diameter. Values of the feed hematocrit are shown at the right of each curve. From Reinke et al. [34] by permission.

be analyzed by examining the radial force balance between forces tending to push cells radially either inward or outward. Studies by Goldsmith and coworkers have shown that deformable particles such as liquid droplets at low concentrations are deformed into ellipsoids by the fluid stresses and migrate toward the tube axis [20]. Other studies have shown that the rate of migration increases with particle size, particle deformability, velocity gradient, and radial distance from the tube axis [21]. Offsetting the forces of axial migration towards the center of the tube or vessel are the dispersion forces such as shear-induced particle diffusion caused by the increased cell–cell and cell–wall interactions that accompany shear flow.

In the absence of aggregation and depending on flow rate and vessel diameter, the velocity profile approximates that of Poiseuille flow, being parabolic and a linear variation of shear rate with radial distance from the wall to the axis. When aggregates are present and migrate towards the center of the tube, the velocity profile is blunted from the parabolic, since the higher viscosity fluid in the center of the stream reduces the shear rate in that region. Conversely, the shear rate near the wall is higher than in Poiseuille flow. When human blood is subject to varying flow rates the dependence of the velocity profile on flow rate is clearly evident [34].

When aggregates migrate to the center of the tube, particle size will be greater in the center of the tube than near the wall and effective viscosity will be greater there as well. The net effect of aggregation on effective blood viscosity in tube flow would be due to two opposing tendencies, increased viscosity in the center due to increased particle size and decreased viscosity near the wall due to reduced hematocrit in that region. In studies on human blood in small glass tubes whose length was from 100 to 1000 times diameter, axial migration was allowed to become fully developed. In this instance a reduction in flow rate allowed larger aggregates to form but the axial migration of these aggregates caused formation of a cell-poor layer near the wall. As a consequence, as shown in Fig. 3 for a 59 μm diameter tube, there was almost no change in apparent viscosity as flow was reduced. In a subsequent report [34] it was shown that increasing aggregation tendency by addition of high molecular weight dextran actually led to a reduction of effective viscosity at reduced flow as the low viscosity of the cell poor region near the wall more than offset the increased viscosity of the aggregates near the center of the tube. These findings were obtained in vertically oriented tubes; in horizontally oriented tubes viscosity increased at low flows due to red cell sedimentation and the absence of a cell-free layer in the lower region of the tube.

Mathematical models have also shown that formation of a cell-free layer at the wall can lead to the shear-independence of blood viscosity in the presence of aggregation as demonstrated in Fig. 3. A model

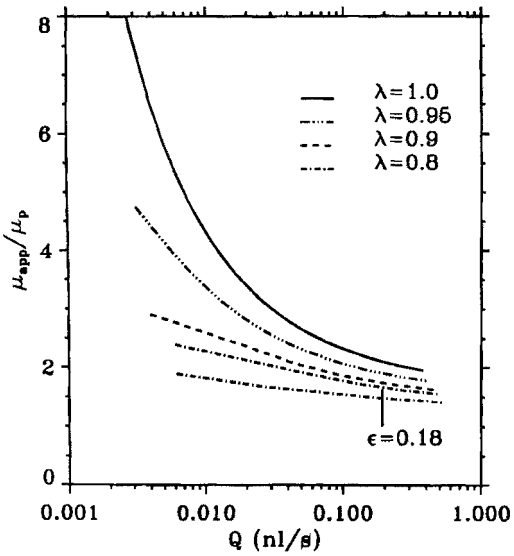

Fig. 4. Relative apparent viscosity as a function of flow rate for the Casson model of blood flow for different red blood cell relative core thicknesses λ ($= r_{\mathrm{RBC}}/R$, $R =$ tube radius) at an eccentricity $\varepsilon = 0.18$, as defined in Das et al. [15].

of tube flow developed by Das et al. [15] based on the Casson model for blood is shown in Fig. 4. When the cell free layer is large (20% of the tube radius) blood viscosity is almost independent of flow rate. However, as the width of the red cell column approaches that of the tube and the cell-free layer almost disappears, blood viscosity becomes much more dependent on flow rate, resembling the relationship seen in Fig. 1. The latter observation may be useful in understanding flow behavior of blood in the venular network.

5. *In vivo* microcirculatory studies

5.1. Vascular morphometry

The vascular network consists of a highly branched system with both series and parallel connections. The frequent branching of arterial and venous networks leads to a ratio of vessel diameter to length, which is much lower than the value of 100 : 1 to 1000 : 1 for tubes used for *in vitro* studies. A study by Engleson et al. in rat spinotrapezius muscle reported a ratio of length to diameter per vascular order of about 10 to 1 [17]. However, since there are generally 2–3 junctions within a vessel of a given order, the ratio of vessel segment length to diameter is considerably lower. In our recent study [7] we found a segment length: diameter ratio of about 3.5 to 1 in this same muscle. This difference in length to diameter ratio between the vascular network and the tubes used for *in vitro* studies has implications for the flow behavior of blood *in vivo* as compared to the single tube studies *in vitro*. One aspect is the effect of red cell aggregation on axial migration *in vivo*.

5.2. Axial migration in vivo

As noted above, the shear rate gradient creates a force, which counteracts dispersion forces and tends to move red cells and aggregates away from the vessel wall. The net effect of this force *in vivo* depends

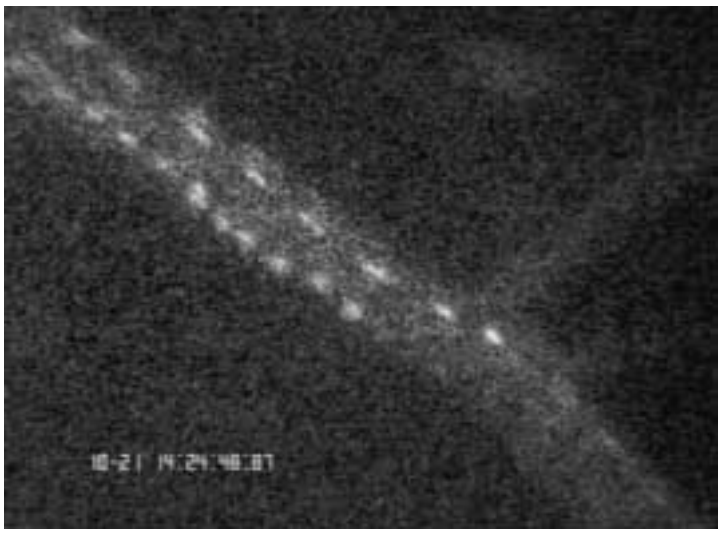

Fig. 5. Video image of fluorescently labeled red cells in a venule of rat spinotrapezius muscle with a gated image intensifier. Shown are multiple images of individual cells due to multiple openings of the gate during a single video frame. Recording such images on successive frames permitted determination of the trajectory of individual red cells during passage through the network.

on the distance over which this process can take place. Using fluorescently labeled red cells injected into the circulation of the rat [6], we were able to follow the trajectories of individual cells in the venular network. An example is shown in Fig. 5. The rat preparation used in these studies serves as a useful model since rat blood does not exhibit significant red cell aggregation under normal conditions but can be induced to aggregate upon addition of high molecular weight dextran. In our studies, dextran 500 was infused to achieve a concentration of 0.6% in plasma. To obtain a range of blood flows in venules, the arterial pressure of the rat was lowered briefly (3–5 minutes) from the control level of 120–130 mm Hg to about 50 mm Hg by removing blood from the animal. At normal arterial pressure the pseudo-shear rates averaged 60–80 s^{-1} and the rate of axial migration (radial movement/longitudinal movement) for a cell near the vessel wall was <1% and was unaltered by dextran infusion, presumably because the shear rates were too high to permit aggregate formation. At reduced arterial pressure the pseudo-shear rates averaged 6–8 s^{-1} and the rate of axial migration in the presence of dextran increased only slightly to 1.3% [7]. This degree of axial migration would ultimately be expected to lead to the formation of a small cell-free layer at the vessel wall. However, due to the branching nature of the vascular network, the segment length between junctions is short as noted above, and there is frequent infusion of red cells and aggregates into the peripheral region of the flow stream, retarding such an effect. Formation of a cell-free layer at the periphery of the vessel in the venular network could only occur with further reduction of arterial pressure and flow, allowing sufficient time for axial migration of all red cells from the vicinity of the wall. The flow rate at which migration forces become dominant over dispersion forces in the venular network can be obtained by experimentally determining the width of the red cell column as compared to the internal diameter of the venule, as described below.

5.3. Cell-free layer formation in vivo

To determine the flow rate at which axial migration leads to formation of a cell free layer at the vessel wall in the venular network we again employed the rat spinotrapezius muscle preparation but

utilized transillumination in the microscope to observe the width of the red cell column and compare that to the internal diameter of the venule under study [8]. In addition, the microscope was oriented horizontally to allow us to observe flow in venules oriented either horizontally or vertically. Blood flow was reduced by lowering arterial pressure by briefly withdrawing blood from the animal. As shown in Fig. 6, in venules oriented vertically to avoid sedimentation, there is clear evidence of a difference in the width of the red cell column and the vessel internal diameter when the pseudo-shear rate was reduced to $< 5\ s^{-1}$. Upon further reduction of arterial pressure, the diameter of the red blood cell column decreased sharply down to a value as low as 65% of control at a pseudoshear rate of 1–2 s^{-1}, similar to values obtained in studies in glass tubes [34]. When the vessel was oriented horizontally to allow sedimentation to occur, the results were essentially identical to those shown in Fig. 6 with the exception that the red cell column was displaced toward the lower wall of the vessel, reflecting the effect of gravity. Since as noted earlier, sedimentation in glass tubes increases effective viscosity [34], a similar effect might be expected here.

Previous *in vitro* studies in long glass tubes [1–3,34] indicated that several seconds are required for the significant development of a cell-free layer at the vessel wall. At a control flow rate of $\sim$5 mm/s in a 50 μm venule (pseudo-shear rate = 100 s^{-1}) with a segment length of 200 μm, blood would travel between bifurcations in 40 ms. With a pseudo-shear rate of 5 s^{-1}, blood would travel between bifurcations in 0.8 s. Apparently this time is sufficient for a cell-free layer to form at the wall. At normalized velocities of 1–2 s^{-1}, blood would traverse a 200 μm distance between bifurcations in about 4–2 s. Based on these observations, it appears that the frequent bifurcations in the venular network infuse red blood cells into the flow stream, especially into the peripheral regions, and effectively retard the development of a cell-free layer until normalized flow rates are reduced to 5 s^{-1} or less. Studies of flow rates in cat sartorius muscle [23] as well as the present studies of the rat spinotrapezius muscle indicate that such flow rates are not seen until arterial pressure is reduced below 40 mm Hg.

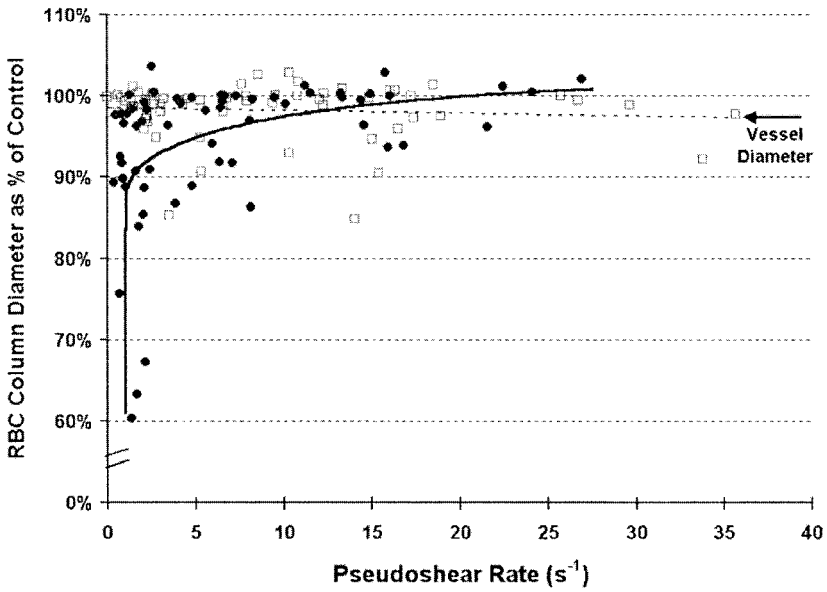

Fig. 6. Diameter of the red cell column of aggregating blood with reduction in arterial pressure and flow. Data shown are for the reduced flow state. The 100% value refers to the control at normal arterial pressure. Also shown is the average vessel diameter at reduced arterial pressure. From Bishop et al. [9] by permission.

5.4. Effect of red cell aggregation on velocity profiles in vivo

Using fluorescently labeled red cells injected into the circulation of the rat as described above, we were able to obtain red cell velocity profiles for venules. Figure 7 shows a characteristic set of velocity profiles for a 60 μm venule under normal and reduced flow rates without aggregation and after induction of aggregation by infusion of dextran 500 into the animal. In order to objectively compare the experimentally-obtained profiles, a linear least squares regression algorithm was used to fit each profile to the equation:

$$V(r) = V_{\max}\left(1 - \left|\frac{r}{R}\right|^{K}\right), \tag{1}$$

where $V(r)$ is the velocity at radial position r, $V_{\max}$ is velocity in the center of the vessel, R is the internal radius of the vessel and the vertical bars denote the absolute value. This equation satisfies the no-slip boundary condition at the vessel wall and has been used previously by Tangelder et al. [35] to describe velocity profiles in rabbit arterioles. The exponent, K, is a measure of the parabolic nature of the profile with $K = 2$ for a parabola and $K > 2$ for a blunted profile. From the set of profiles shown in Fig. 7, it can be seen that under non-aggregating conditions, the velocity profiles at both high and low flow rates are parabolic in nature as would be expected for a Newtonian fluid ($K = 2$). However, after

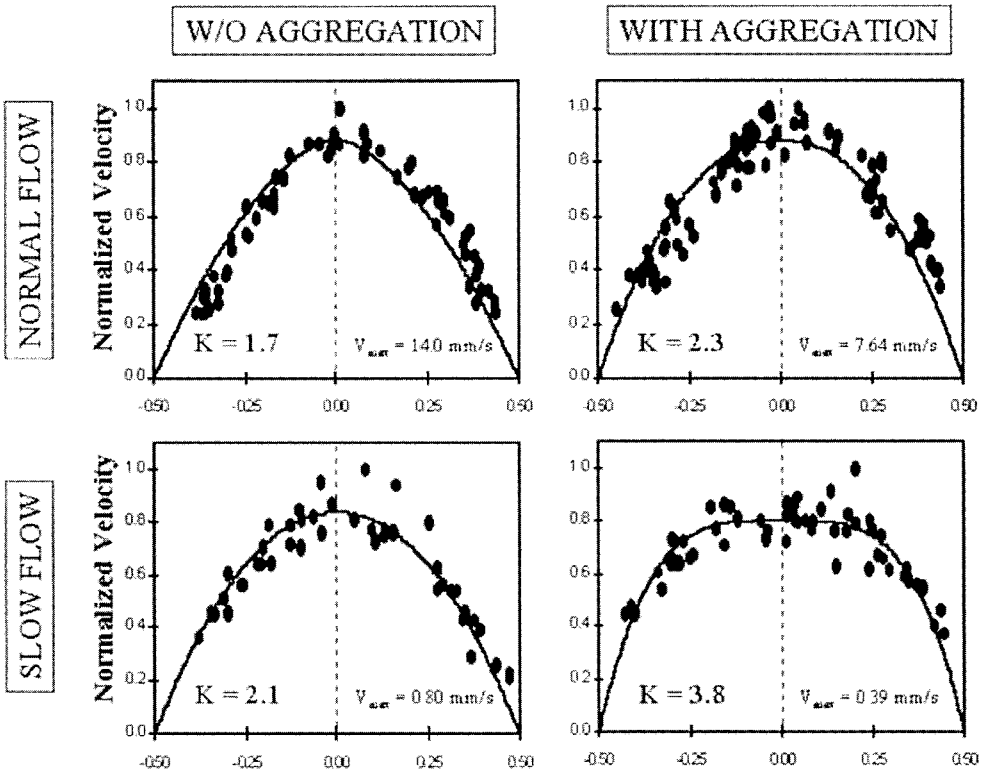

Fig. 7. Velocity profiles at normal and reduced flow with and without red cell aggregation obtained with fluorescently labeled red cells and the technique shown in Fig. 4. K values refer to exponent shown in Eq. (4). From Bishop et al. [7] by permission.

induction of red cell aggregation, the velocity profile becomes blunted ($K > 2$) from the parabolic shape seen for the non-aggregating blood. This blunting is particularly evident at the lower flow rates.

Velocity profiles from venules in a number of different animals were obtained, and the exponent, K, plotted against red cell velocity in Fig. 8. It can be seen that a significant difference exists between the velocity profiles of control and dextran-treated animals below a pseudo-shear rate of 40 s^{-1} and may be present at pseudo-shear rates as high as 90 s^{-1} where the regression lines for normal and dextran-treated vessels intersect. This value is larger than the control pseudo-shear rate in many areas of the circulation even on the arterial side, indicating that red cell aggregation may influence blood rheology in more areas of the circulation than was previously thought.

The findings from microcirculatory studies described above may aid in explaining changes in venous resistance shown in Fig. 1. Using the values for K of the velocity profiles shown in Fig. 7 we estimated the shear stress at the venular wall from differentiation of Eq. (1) above [6]. Based on studies of human blood in rotational viscometers [11,12] we assumed that the viscosity of blood near the wall is independent of flow rate. On this basis we find that the effective viscosity of the blood in venules of about 60 μm internal diameter may be doubled at the lowest shear rates by red cell aggregation. The aggregation induced in these studies as indicated by the average red cell sedimentation rate (ESR) of 8.0 mm/hr is somewhat greater than that found in the cat (5.4 mm/hr) and the dog (3.5 mm/hr) but less than that seen in humans (10.7 mm/hr) [6]. Thus it appears that aggregation may explain a portion of the increase in venous vascular resistance seen with reduced flow in the studies on dogs and cats. As noted above, theoretical studies by Das et al. [15] of the contribution of red cell aggregation using the Casson model for the flow properties of aggregating blood also predict that in the absence of a cell-free peripheral layer, aggregation would increase venous resistance substantially with flow reduction. Venules in cat skeletal

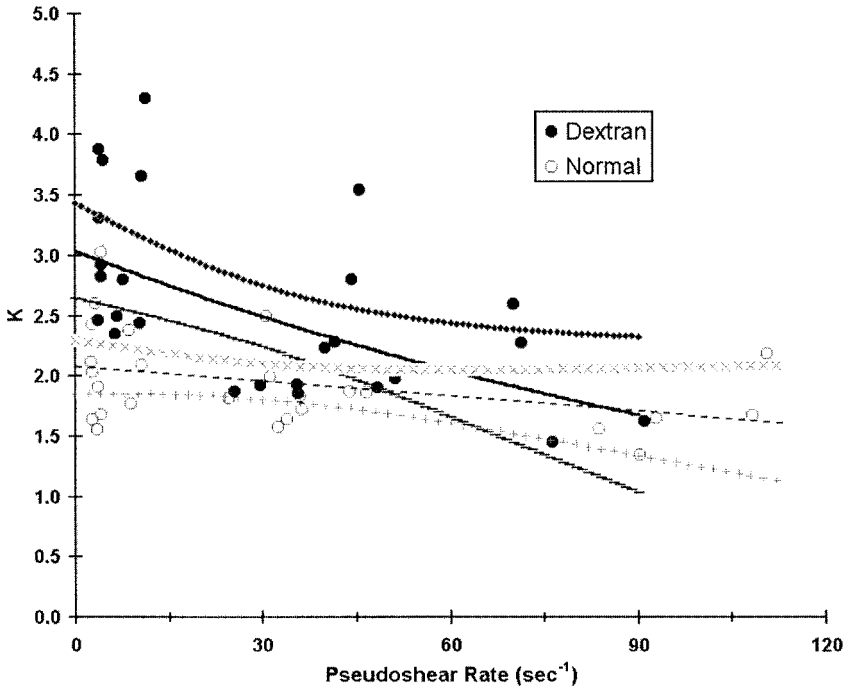

Fig. 8. K values for velocity profiles in venules with normal rat blood and blood to which dextran 500 has been added to achieve a concentration of 0.6% in plasma approximately. From Bishop et al. [7] by permission.

muscle during arterial pressure reduction undergo only a very small change in horizontal diameter [23] and similar small changes in horizontal and vertical diameter were seen in venules of rat skeletal muscle by [5], indicating that dimensional changes in the venules are unlikely to contribute significantly to the rise in venous resistance.

In addition to an effect on the velocity profile, aggregation will displace white cells from the center of the flow stream, causing margination and increasing the likelihood of white cells rolling along the vessel wall or adhering to it and in the process significantly increasing resistance to flow [16,19]. Pearson and Lipowsky [31] found that aggregation induced by dextran 500 increased the fraction of white blood cells rolling along the venular endothelium and adhering at wall shear rates below 350 s^{-1}. Taken together, the effects of aggregation on the velocity profile and on leukocyte margination may account for the inverse relationship between venous resistance and blood flow reported in skeletal muscle of dogs and cats and may also be important in other "athletic" species that possess the property of red cell aggregation.

It is important to note that comparison of the effects of red blood cell aggregation on blood rheology in rotational viscometers, in small glass tubes, and in the venular network as described here reveal that the geometry of the system is an extremely important determinant of the flow behavior of blood. Blood flowing through relatively short tubes appears to behave in some respects quite differently from that seen in a single long tube due to the absence of a cell-poor or cell-free layer at the wall. Conversely, the effect of shear rate on blood viscosity in the venular network appears somewhat similar to that seen in rotational viscometers. It appears that a very detailed examination of the flow patterns *in vivo* is required before we can determine how findings from *in vitro* systems can be usefully applied to understanding the flow properties of blood *in vivo*.

Acknowledgments

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