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Hemorheology and vascular control mechanisms

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Abstract. Blood rheology is a well-known determinant of tissue perfusion and, according to the Poiseuille relation, hemodynamic resistance in a constant-geometry vascular network is directly proportional to blood viscosity. However, this direct relationship cannot be observed in all *in vivo* studies. Further, there are several reports indicating marked differences between the *in vivo* and *ex vivo* flow properties of blood. These differences can be explained, in large part, by considering special hemorheological mechanisms (e.g., Fahraeus–Lindqvist effect, axial migration) that are of importance in the microcirculation. Additionally, the influence of altered rheological properties of blood and its components on vascular control mechanisms requires consideration: (1) There is an indirect relation between blood rheology and microvascular tone that is mediated by tissue oxygenation, with a compensatory vasodilation occurring if tissue perfusion is impaired due to hemorheological deterioration; (2) Blood rheology may influence vascular tone through alterations of wall shear stress, which in turn determines endothelial generation of vasoactive substances (e.g., nitric oxide). This latter point is of particular relevance to the field of clinical hemorheology, since enhanced red blood cell aggregation has been shown to affect nitric oxide synthesis and thus control of vascular smooth muscle tone. Such multiple pathways by which hemorheological changes can affect vascular resistance help to explain the continuing difficulty of predicting correlations between *in vivo* and *ex vivo* hemorheological behavior; they also suggest the need for continued experimental studies in this area.

Keywords: Vascular resistance, viscosity, red blood cell, rheology

1. Introduction

The resistance to flow of simple fluids through rigid cylindrical tubes is known to be determined by geometrical and rheological factors, with the Poiseuille equation indicating that flow resistance is inversely proportional to the fourth power of radius and directly proportional to the length of the tube and the viscosity of the fluid. Although not always strictly correct, the Poiseuille equation has generally been applied to blood flow in vessels. Since the radius appears as the fourth power, vessel size was considered to be the most important determinant of flow resistance, with the other factors almost totally ignored. The major influence of cellular pathology theory on medical practice furthered this position: vascular geometry became the only important factor, with the diagnosis and understanding of most diseases based on the microscopic examination of dead and fixed tissue samples [4]. It is thus understandable why a functional parameter, such as the fluidity of blood, was previously not recognized as an important factor in medical practice but rather was considered to be no more than another "constant" in the Poiseuille equation.

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In the first half of the twentieth century, the work of Robin Fahraeus established a bridge between the very long medical tradition of humoral pathology and modern hemorheological concepts [18,27]. In addition to his pioneering work on the suspension stability of blood [23], he was the first to observe the uneven distribution of blood flow in the cross-section of blood vessels [22], and thus made one of the first *in vivo* hemorheology studies. The results of his studies played a very important role in the establishment of modern hemorheological concepts, and lead to hemorheological parameters being measured in a vast number of pathophysiological conditions [16,33,44]. Cardiovascular diseases, diabetes, and sepsis are among the disease processes with well-established hemorheological profiles [16,33]; significant hemorheological alterations have also been reported in extreme physiological states such as exhaustive exercise [51]. In addition to clinical studies, there are reports indicating hemorheological changes in animal models of pathologic states (e.g., [3,7,38,49]).

2. The importance of hemorheological alterations

While there now exists a very large collection of clinical and experimental studies reporting "*statistically significant*" alterations of hemorheological parameters in a wide variety of conditions, the importance of these alterations from a pathophysiological point of view has not yet been fully explored. Hemorheological alterations might have two distinct implications: (1) Hemorheological parameters might be altered due to pathophysiological processes, since it is well known that hemorheological parameters are sensitive to body homeostasis. For example, maintenance of normal red blood cell deformability depends on intact metabolic pathways [36], the proper microenvironment and "good cellular neighbors". Activated white blood cells have been demonstrated to induce impaired deformability in neighboring red cells [5]; (2) Altered hemorheology may play a role in the development of pathophysiological processes. A classic example of this type of relationship is sickle cell disease in which clinical signs and symptoms can be related to abnormal RBC rigidity. There are also genetic models of hypertension with red cell abnormalities [10,39], and it is possible that such alterations may play a role in the elevation of blood pressure by affecting peripheral vascular resistance [32].

The two abovementioned approaches to hemorheology-disease relationships may reflect a vicious cycle or, perhaps more correctly, a "chicken versus egg" dilemma. However, the two aspects of the cycle are not equally well established. That is, while there is a close relationship between homeostasis and hemorheology, and it is well known that general or local homeostasis can affect hemorheological factors, the role of hemorheology in maintaining homeostasis is not equally well defined. One factor contributing to this inequality are experimental studies indicating that the fluidity of blood *in vivo* might be quite different than that predicted based on measurements made outside of the vascular system.

3. Behavior of blood *in vivo*

Probably the most well-known study of the *in vivo* rheological behavior of blood is the Whittaker and Winton paper of 1933 [50]. In this publication they reported that the apparent viscosity of blood *in vivo* was lower than that measured via viscometry *in vitro* [50]; several other publications have reported similar results [6,14,20]. Such findings thus suggest difficulties in predicting the exact degree of alteration of tissue perfusion due to a given degree of hemorheological abnormality: flow resistance *in vivo* is more

complex than predicted based solely on *ex vivo* rheological data. Note that the apparent discrepancy between *in vitro* and *in vivo* results [50] can be explained, in part, using the concepts developed by Robin Fahraeus [27]: axial migration of deformable red blood cells during flow and its consequences such as the Fahraeus effect (i.e., reduced hematocrit at the microvascular level) may explain the lower apparent viscosity *in vivo* [17,40,43].

4. The role of vasomotor control in determining blood flow resistance

In addition to factors related to the rheological behavior of blood, factors related to blood vessels should also be considered when attempting to explain the difference between *in vivo* and *ex vivo* flow resistance. That is, blood vessel geometry and orientation are important determinants of the effects of hemorheological alterations on flow resistance [1,11,17,41,43,46]. Additionally, the role of vasomotion should also be considered, since vasomotion alters vessel radius and hence has a direct influence on vascular hindrance. Therefore vascular hindrance should be considered as a variable when exploring pressure–flow relationships of the vascular system, and not merely as an important but constant determinant of flow resistance.

Vascular hindrance is primarily affected by vessel radius, and hence is regulated by factors that affect vascular smooth muscle tone. While a variety of factors play role in this regulation, the most important is the metabolic demands of the perfused tissues [21,45]. Additionally, mechanical forces acting on the endothelium also influence smooth muscle tone and vascular hindrance [13,15].

4.1. Metabolic autoregulation

In general, autoregulation refers to the intrinsic ability of a vascular bed or organ to maintain blood flow at control levels in spite of changes of perfusion pressure; a logical extension of this definition would include challenges due to altered hemorheological factors. Autoregulation due to metabolic factors is a very effective mechanism for maintaining blood flow: a decrement in blood flow to a given tissue and related hypoxia can be normalized by decreased vascular hindrance even if the main cause of the blood flow decrement still continues to exist. The key element for this regulation is decreased tissue oxygen partial pressure due to insufficient blood flow [21,24,45], with the decrease being the result of decreased arterial pressure or decreased blood fluidity; the latter challenge can be considered to be a hemorheological “extra load”.

The role of vasomotion in determining flow resistance alterations induced by a hemorheological extra-load can be demonstrated experimentally. In a yet unpublished study, an isolated-perfused rat hind-limb preparation was used to investigate the role of vasomotor control in the response to hemorheological changes. The left femoral artery and femoral vein of rats were cannulated and the isolated hind limb preparation perfused using a pressure servo-control system (Fig. 1): hydrostatic pressure was monitored at the entrance of the arterial catheter and the speed of the roller pump (i.e., flow rate) adjusted to maintain arterial pressure at the set point (i.e., 100 mmHg), with flow resistance calculated based upon the measured pressure and flow rate. RBC-autologous plasma suspensions, at 0.4 l/l hematocrit, were prepared using either normal or chemically-rigidified rat red blood cells. The deformability of rat red cells was reduced, in a graded fashion, by incubating them with glutaraldehyde at concentrations between 0.0005–0.002%. At these low concentrations the decrease of deformability was subtle: RBC deformability measured at a low shear stress (1.58 Pa) was decreased by only about 16% at the highest glutaraldehyde concentration (Fig. 2A).

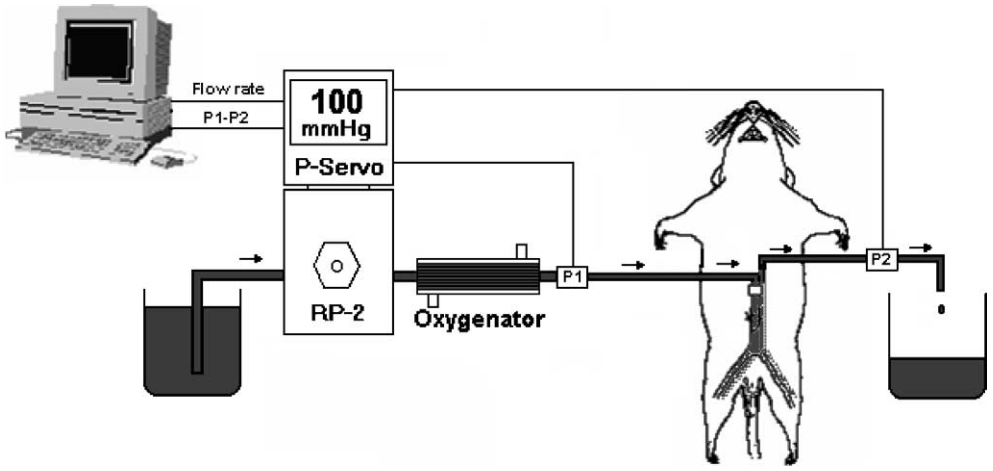

Fig. 1. Schematic representation of the experimental set up for determining blood flow resistance in isolated-perfused rat hind limb. P1 and P2 are pressure transducers, and P-Servo is the pressure servo controller device that maintains the pressure at P1 at 100 mmHg by automatically adjusting the flow rate generated by the roller pump (RP-2). Normal or glutaraldehyde treated rat RBC suspensions in autologous plasma were used for perfusion.

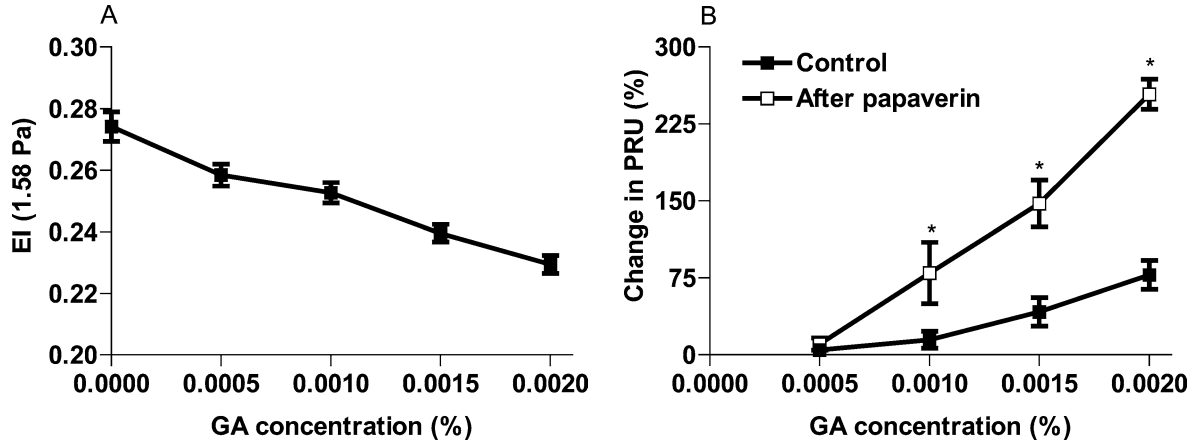

Fig. 2. Effect of glutaraldehyde concentration on RBC elongation index (i.e., deformability) as measured by ektacytometry at a shear stress of 1.58 Pa (panel A); Increase of blood flow resistance, relative to control (i.e., normal, untreated RBC), during perfusion with 0.4 l/l hematocrit glutaraldehyde-treated RBC suspensions (panel B). Data are mean $\pm$ standard deviation; $n = 4$ in both groups. *: Difference from control, $p < 0.001$.

In hind limb preparations with intact vasomotor control, infusions of red cell suspensions of decreasing deformability resulted in gradual increments of flow resistance (Fig. 2B). The increment in flow resistance during perfusion with the suspension of RBC treated with the highest glutaraldehyde concentration was about 78 percent. When vascular smooth muscle tone was inhibited by adding 10^{-4} M papaverin to the RBC suspensions being used, seminal observations included: (1) the absolute values of flow resistance markedly decreased (data not shown); (2) the change in flow resistance due to altered RBC deformability was significantly greater compared to preparations with intact vascular control mechanisms (Fig. 2B).

The experiment results shown in Fig. 2 clearly demonstrate the importance of vasodilatory reserve that can be used to compensate for alteration in tissue perfusion due to abnormal rheological behavior of blood (i.e., due to red cell deformability impairment in the example presented above). Nature also does such "experiments" by changing the geometry of blood vessels by pathological processes and thereby exhausting the vasodilatory reserve. In this case, any alteration that causes a decrease in blood flow (e.g., blood viscosity increment) cannot be compensated in the affected vascular network, whereas compensation can occur in vascular networks with sufficient autoregulatory reserve.

In another experiment series designed to demonstrate the importance of autoregulatory reserve in compensating for a hemorheological extra load, dogs were prepared by cannulating major blood vessels and the coronary sinus, and an electromagnetic flow probe was placed on the left coronary artery [8]. The dogs were then randomly assigned to one of two groups and a critical stenosis was applied to the left coronary artery of one group. The critical stenosis was applied using an adjustable mechanical occluder positioned proximally to the flow probe: (1) the artery was first constricted in small steps until a drop in coronary flow rate was just detected by the flow probe; (2) the occluder was than opened one step in order to achieve the flow rate that existed just before the flow rate reduction occurred – at this point the degree of narrowing of the artery varied between animals but was always greater than 70 percent constriction. The hematocrit of the dogs was increased about 50 percent by isovolemic exchange transfusion of packed dog red cells, resulting in about 100% increment in blood viscosity. Arterial and right atrial pressures and coronary artery blood flow were recorded before and after the hematocrit change.

As shown in Fig. 3, coronary artery resistance only slightly and non-significantly increased after the hematocrit was increased in the group with normal left coronary arteries (Fig. 3, left portion). However, in animals with stenosed left coronary arteries, flow resistance was significantly elevated after the same increase of hematocrit (Fig. 3, right portion). Additionally, erythrocyte deformability measured in blood samples obtained from coronary sinus (i.e., the blood returning from the coronary microvasculature) was more impaired if the left coronary arteries were critically stenosed (Fig. 4). Thus, changes of both flow resistance and RBC deformability in response to a hemorheological over load depend critically on the degree to which the coronary arteries are geometrically challenged [8].

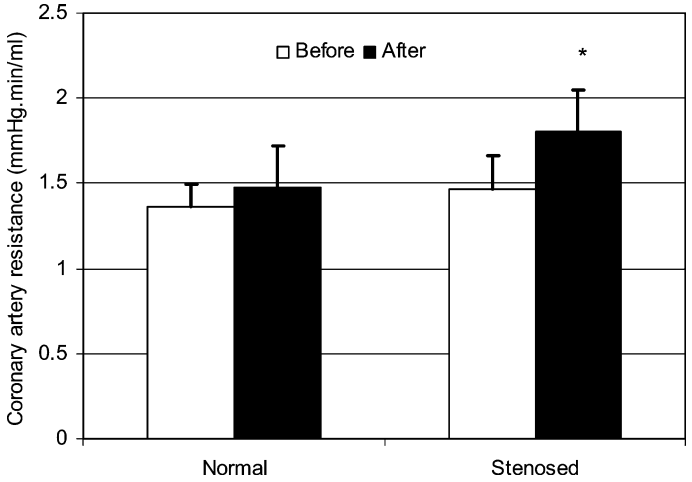

Fig. 3. Coronary artery resistance of dogs with normal or stenosed left coronary arteries before and after a 50% increment of hematocrit. Data are mean $\pm$ standard error; $n = 10$ for each group; *: Difference from the value obtained before the exchange transfusion, $p < 0.05$. (Redrawn from [8].)

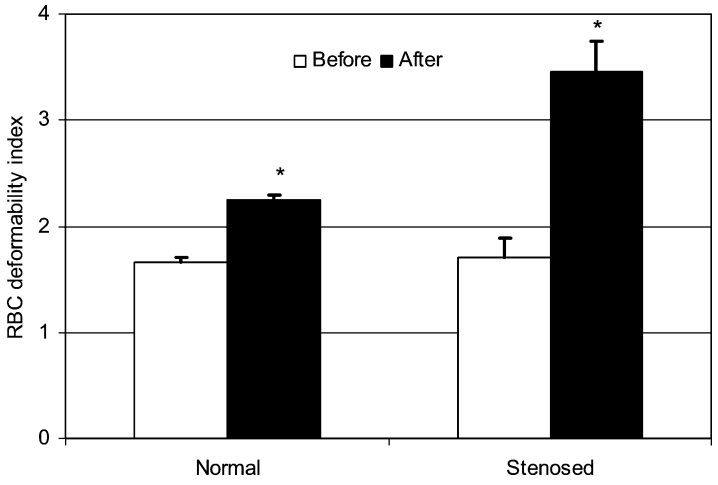

Fig. 4. RBC deformability indexes measured in blood samples obtained from the coronary sinus of dogs with normal or stenosed left coronary arteries before and after a 50% increment of hematocrit. Data are mean ± standard error; $n = 10$ for each group; *: Difference from value obtained before the exchange transfusion, $p < 0.05$. (Redrawn from [8].)

4.2. The effect of shear forces on vascular control mechanisms

Vascular tone is not only controlled by local metabolic factors, but also by specific vasoactive substances [12,42], with the most notable of these being nitric oxide [28]. Nitric oxide (NO) is a simple molecule that is synthesized in endothelial cells and diffuses to underlying smooth muscle cells where it activates guanylate cyclase [19,28,42]; the resulting increment in cyclic GMP results in smooth muscle relaxation [19,28]. NO is synthesized by NO synthases (NOS) using L-arginine as the substrate [31]. Endothelial NOS, which plays a major role in the regulation of vascular tone, is control by a variety of factors including hypoxia and shear stress [31]. These factors not only determine the activity of the existing enzyme, but also affect the expression of eNOS protein in endothelial cells [26,31]. Mechanical forces acting on endothelial cells are known to be an important determinant of the cell's NO output [25,47], with tangential wall shear stress affecting the NO synthesis mechanisms of endothelial cells [34,37]. Wall shear stress is, in turn, determined by the fluid velocity near the vessel wall and the viscosity of the fluid in this region [34,37]. Note that decreased wall shear stress down regulates NO output and may result in increased smooth muscle tone and hence increased vascular hindrance [48].

There are several experimental studies confirming the role of fluid velocity (i.e., local fluid shear forces) in determining NO output from vascular endothelium and hence flow-induced vasodilation mediated by NO (e.g., [29,30,35,48]). Using a rat chronic heart failure model induced by left coronary artery ligation, Varin et al. [48] indicate that flow-induced dilation in gracilis muscle arteries in response to various levels of fluid flow was totally abolished in vessels from rats with chronic heart failure; this response is well known to be mediated by NO. Varin et al. also demonstrated that NOS expression in skeletal muscle was suppressed in chronic heart failure. Interestingly, both the lack of flow-induced dilation and the suppression of NOS in rats with chronic heart failure could be prevented by a swimming exercise protocol that intermittently increased cardiac output and blood flow in skeletal muscle vessels [48].

A recent paper by Miyauchi et al. [35] also presents evidence supporting the effects of fluid forces on endothelial NO activity. In their study, rats were subjected to heavy exercise for 45 minutes, following which NOS activity was determined in the lungs and kidneys of these animals. Note that lung represents an organ with increased blood flow during exercise, while kidney represents the opposite (i.e., an organ with reduced blood flow during exercise). Their results indicated that compared to non-exercising animals, rats subjected to exercise had increased total NOS activity in their lungs but decreased total NOS activity in their kidneys, with the changes related to constitutive NOS activity [35]. The two examples cited above [35,48] thus clearly show that if wall shear stress is altered due to altered blood flow then NO synthesizing mechanisms are affected.

In addition to the blood flow rate near the vessel wall, the viscosity of the fluid in this region also affects wall shear stress [34,37]. It is well known that the composition, and hence the fluidity, of blood flowing in a cylindrical blood vessel is not uniform throughout the cross-section [17,27]. Rather, the distribution of RBC is not uniform and is influenced by axial migration [17]; increased RBC aggregation results in enhanced axial accumulation of red cells [11,22]. In turn, the enhanced accumulation of red cells in the central flow zone leaves a lower hematocrit, less viscous fluid in the marginal zone near the vessel wall [17]. Thus it can be expected that enhanced red cell aggregation may reduce wall shear stress and result in down-regulation of NO synthesis mechanisms and a resulting increase of vascular resistance.

The abovementioned hypothesis regarding the effects of RBC aggregation was tested in an experimental study performed on rats in which RBC aggregation was chronically enhanced by isovolumic exchange transfusions of poloxamer-coated red blood cell suspensions [9]. Poloxamer-coated red cells were suspended in autologous plasma at 0.4 l/l hematocrit and exchange transfused to rats to achieve a 30 percent exchange of the total blood volume. Note that in this approach, poloxamer copolymers are covalently attached to the RBC surface, and by appropriate selection of the poloxamer's molecular weight, RBC aggregation in autologous plasma can be significantly enhanced [2]. Conversely, use of a lower molecular weight poloxamer results in unaltered aggregation and thus provides a suitable control for the strongly aggregating suspensions.

In rats exchange transfused with RBC coated with a poloxamer known to enhance aggregation (F98), the extent of aggregation was found to be increased about 4-fold immediately after the exchange transfusion and to decrease gradually over a 4 day follow-up period [9]. Conversely, aggregation was not altered at any time point in rats exchanged with cells coated with the lower molecular weight poloxamer (F68). Mean arterial blood pressure, measured daily during the follow-up period using a non-invasive method, was found to increase gradually only in the group with enhanced aggregation. Moreover, NO-dependent relaxation responses of resistance artery segments obtained from the gracilis muscle of animals with enhanced RBC aggregation were found to be altered. Figure 5 demonstrates these findings and presents the flow-induced dilation response that is known to be NO mediated: in the group with enhanced red cell aggregation, the maximum dilation response was observed at a higher flow rate and the percentage of maximum dilation was only 50 percent of the control group. These data, supported by results indicating suppressed acetylcholine-induced dilation responses [9], suggest that NO related mechanisms were significantly depressed in the rats with enhanced red cell aggregation; this suggestion was confirmed by data indicating decreased eNOS expression in tissue samples obtained from gracilis muscle [9]. Such studies thus indicate that enhanced red cell aggregation may down-regulate NO-related vascular control mechanism yielding increased vascular resistance and altered blood pressure; thereby suggesting another means by which hemorheological parameters can influence vasomotor control.

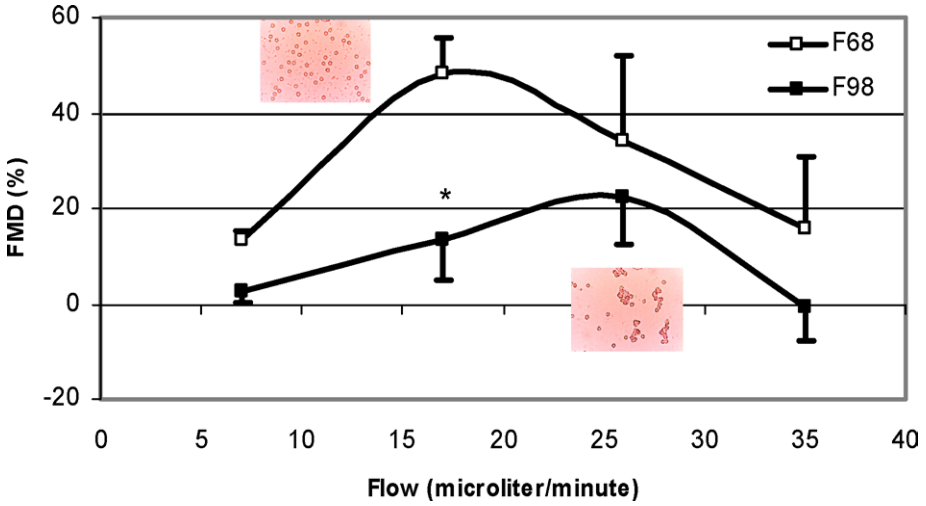

Fig. 5. Flow mediated dilation (FMD) in resistance arteries isolated from the gracilis muscles of rats exchanged with poloxamer-coated RBC. Cells coated with poloxamer F98 exhibit greatly enhanced aggregation while those coated with poloxamer F68 exhibit aggregation unaltered from control. Photographic insets indicate extent of RBC aggregation in blood samples from exchanged animals. Data are mean $\pm$ standard error; $n = 8$ for each group. (Redrawn from [9].)

5. Conclusion

The rheological behavior of blood is not merely a factor that, when combined with vascular hindrance (i.e., vessel geometry), determines hemodynamic resistance. Rather, hemorheological properties can also determine the magnitude of vascular hindrance via processes leading to altered vessel geometry. The effects of rheologic changes are probably mediated by a variety of vascular control mechanisms, and most likely include such phenomena as metabolic autoregulation and shear stress induced NO synthesis by vascular endothelium. Such multiple pathways by which hemorheological changes can affect vascular resistance may thus explain, in part, the continuing difficulty of predicting correlations between *in vivo* and *ex vivo* hemorheological behavior; they also suggest the need for continued experimental studies in this area.

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