Blood Rheology and Hemodynamics

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ABSTRACT

Blood is a two-phase suspension of formed elements (i.e., red blood cells [RBCs], white blood cells [WBCs], platelets) suspended in an aqueous solution of organic molecules, proteins, and salts called plasma. The apparent viscosity of blood depends on the existing shear forces (i.e., blood behaves as a non-Newtonian fluid) and is determined by hematocrit, plasma viscosity, RBC aggregation, and the mechanical properties of RBCs. RBCs are highly deformable, and this physical property significantly contributes to aiding blood flow both under bulk flow conditions and in the microcirculation. The tendency of RBCs to undergo reversible aggregation is an important determinant of apparent viscosity because the size of RBC aggregates is inversely proportional to the magnitude of shear forces; the aggregates are dispersed with increasing shear forces, then reform under low-flow or static conditions. RBC aggregation also affects the in vivo fluidity of blood, especially in the low-shear regions of the circulatory system. Blood rheology has been reported to be altered in various physiopathological processes: (1) Alterations of hematocrit significantly contribute to hemorheological variations in diseases and in certain extreme physiological conditions; (2) RBC deformability is sensitive to local and general homeostasis, with RBC deformability affected by alterations of the properties and associations of membrane skeletal proteins, the ratio of RBC membrane surface area to cell volume, cell morphology, and cytoplasmic viscosity. Such alterations may result from genetic disorders or may be induced by such factors as abnormal local tissue metabolism, oxidant stress, and activated leukocytes; and (3) RBC aggregation is mainly determined by plasma protein composition and surface properties of RBCs, with increased plasma concentrations of acute phase reactants in inflammatory disorders a common cause of increased RBC aggregation. In addition, RBC aggregation tendency can be modified by alterations of RBC surface properties because of RBC in vivo aging, oxygen-free radicals, or proteolytic enzymes. Impairment of blood fluidity may significantly affect tissue perfusion and result in functional deteriorations, especially if disease processes also disturb vascular properties.

KEYWORDS: Hemorheology, hemodynamics, viscosity, erythrocyte deformability, erythrocyte aggregation, tissue perfusion, blood flow

Objectives: On completion of this article the reader should be able to (1) describe the manner in which blood viscosity is affected by hematocrit, shear rate, and red cell aggregation; and (2) indicate the importance of red cell deformability and list factors which affect this cellular mechanical property.

The Hyperviscosity Syndromes; Editor in Chief, Eberhard F. Mammen, M.D.; Guest Editor, Hau C. Kwaan, M.D., Ph.D. *Seminars in Thrombosis and Hemostasis*, volume 29, number 5, 2003. Address for correspondence and reprint requests: Herbert J. Meiselman, Sc.D., Department of Physiology and Biophysics, University of Southern California, Keck School of Medicine, 1333 San Pablo Street, MMR 626, Los Angeles, CA 90089. E-mail: meiselma@hsc.usc.edu. [1]Department of Physiology, Akdeniz University Faculty of Medicine, Antalya, Turkey, and [2]Professor, Department of Physiology and Biophysics, University of Southern California School of Medicine, Los Angeles, California. 0094–6176,p;2003,29,05, 435,450,ftx,en;sth00907x.

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HISTORICAL PERSPECTIVES

Prior to the realization of the cellular structure of living material, all medical theories and practice were based on the concept of "humors."[1] The concept of "humors" was, in turn, a direct application of Greek natural philosophy to medicine. Hippocrates is known as the father of humoral pathology theory. According to this medical theory, the human body contains a well-balanced mixture of four juices (or humors): sanguine, choleric, phlegmatic, and melancholic. Early physicians believed that the imbalance between various humors of the body would cause disease, and treatment should be based on reestablishing this balance. It is interesting to note that the diagnosis of this imbalance was mostly performed by inspecting blood samples from patients and determining the relative amounts of each humor. The melancholic humor was the lowest, dark part of clotting blood; the choleric humor was the serum separating from the clotting blood; and the sanguine humor was represented by RBCs. Phlegmatic humor or juice was accepted to be visible only in the blood of patients and was located on top of melancholic humor, with the amount of this humor directly related to the severity of the disease. We now know that this phlegmatic portion of the blood is, in reality, the "buffy coat" in clotted or sedimented blood and comprises WBCs, platelets, and polymerized fibrinogen.

According to the humoral pathology approach, the standard procedure to reestablish the balance between humors was phlebotomy (i.e., removal of blood from the body).[2] Many physicians recognized that the properties of blood were altered in situations such as inflammation and that this alteration prevented adequate blood flow; phlebotomy helped to restore blood flow. Interestingly, most of these earlier observations were made prior to William Harvey's discovery of the circulation in the 17th century.[3] Herman Boerhaave, who introduced the laws of physics into medical thinking, also enriched the medical ideas of the 17th century.[4] Boerhaave's intravital microscopy studies resulted in a better understanding of blood flow disturbances that were believed to be caused by the imbalance of humors. In the mid-19th century, Poiseuille made significant contributions to physiology and fluid mechanics by observing the flow behavior of fluids in glass capillary tubes and developing the well-known Poiseuille's law for tube flow.[5]

Although humoral pathology ideas were beginning to be based on more scientific concepts towards the end of 19th century, cellular pathology theory was also evolving. Rudolf Virchow was very successful in establishing a new concept of disease that was based on the structural and functional disturbances of cells.[6] These disturbances could be detected under a microscope by observing tissue samples that were fixed and dyed. The influence of Virchow's cellular pathology theory on 20th-century medicine was enormous, with every disease explained by microscopic disturbances observed in dead, fixed tissues. Although highly respected by the medical community, such an approach failed to consider the dynamic nature of living systems and resulted in a highly static view of disease processes. The cellular pathology approach grew very rapidly, especially during the first half of the 20th century, and, parallel to this growth, concepts that were seen as being related to humoral pathology theory were deemed nonscientific. Humoral pathology theory rapidly lost ground to the cellular pathology theory, and even the oldest method of medical treatment with proven value in many patients—hemodilution by various means—was eliminated from the practice of medicine.

During the early part of the 20th century, Robin Fahraeus, a Scandinavian pathologist, began exploring the flow properties of blood.[5,7] He discovered that the suspension stability and fluidity of blood were altered during disease processes, explained the humoral pathology concepts by modern scientific ideas, and provided a basis for understanding medical practices of previous centuries. Fahraeus' ideas of were not widely appreciated until the latter part of the 20th century, although the measurement of blood sedimentation rate, a test that he described, remains one of the most widely used routine laboratory procedures in modern medicine. During the last several decades, the dynamic nature of blood flow and its rheological behavior have begun to be widely investigated. Development of appropriate techniques to study the flow behavior of blood and its components, together with the evolution of modern concepts of fluid dynamics, has thus led to the growth of a new medical field termed blood rheology or hemorheology.

PRINCIPLES OF RHEOLOGY

Rheology is the scientific field that deals with the flow and deformation behavior of materials, with the materials under consideration being solids or fluids, including liquids and gases.[8,9] *Deformation* can be defined as the relative displacement of material points within the body.[10] Solids react to the application of a force by a given deformation. If a solid is elastic, the deformation is pro-

portional to the applied force, and, if the deformation is not too large, the original shape is recovered when the force is removed.[10] If a permanent deformation remains after the removal of force, the solid is said to be plastic. Fluids continuously deform—or flow—because of the application of applied forces.[10] Some materials exhibit viscoelastic behavior, which is a combination of fluid-like and solid-like behavior.[11]

In studying the degree of deformation (or flow) of a material, the force applied per unit area must be considered.[10] This deforming force, termed stress, may have several components, including (1) shear stress, the force per unit area acting parallel to the surface, and (2) normal stress, the force per unit area acting perpendicular to the surface. The latter is defined as pressure in a fluid. The degree of deformation is termed strain, which also has various components associated with the different stress components.[10] For example, shear stress results in shear strain, often termed shear rate, in which the layers of material move parallel to each other in a progressive manner.

Early studies in fluid mechanics revealed that for a pipe of constant diameter and length and for a given fluid, the resistance to flow depended on the flow conditions within the pipe.[9] Experimental data obtained during the second half of the 19th century revealed that during slow flow the pressure drop (reflecting the resistance to flow) was proportional to the speed of flow. Under these conditions, it has been observed that the liquid particles move smoothly in adjacent planes (laminae) parallel to the tube wall; this type of flow is called laminar flow.[12] With increasing flow rate, there is a tendency for the fluid flow to become irregular, with fluid moving in swirls and irregular patterns. This type of chaotic flow is termed turbulent, with the degree of turbulence increasing with flow rate.[13] Under such turbulent conditions, the pressure drop is proportional to the square of the speed of flow, and thus for the same pipe and fluid, resistance to flow is greater with turbulence than it is for laminar flow.

Under laminar flow conditions, a shear stress–shear rate relationship is used to define the fluidity of liquids.[9,10,12] This relationship reflects the internal resistance between fluid layers (laminas) and thus reflects the viscosity of the fluid; the viscosity of a liquid can be calculated by dividing the shear stress by the shear rate.[9,10,12] From a rheological point of view, liquids can be divided into two main groups (Fig. 1). (1) In Newtonian liquids, the viscosity is independent of variations in shear rate or shear stress. For these fluids the slope of the shear stress–shear rate relation is constant over the range of

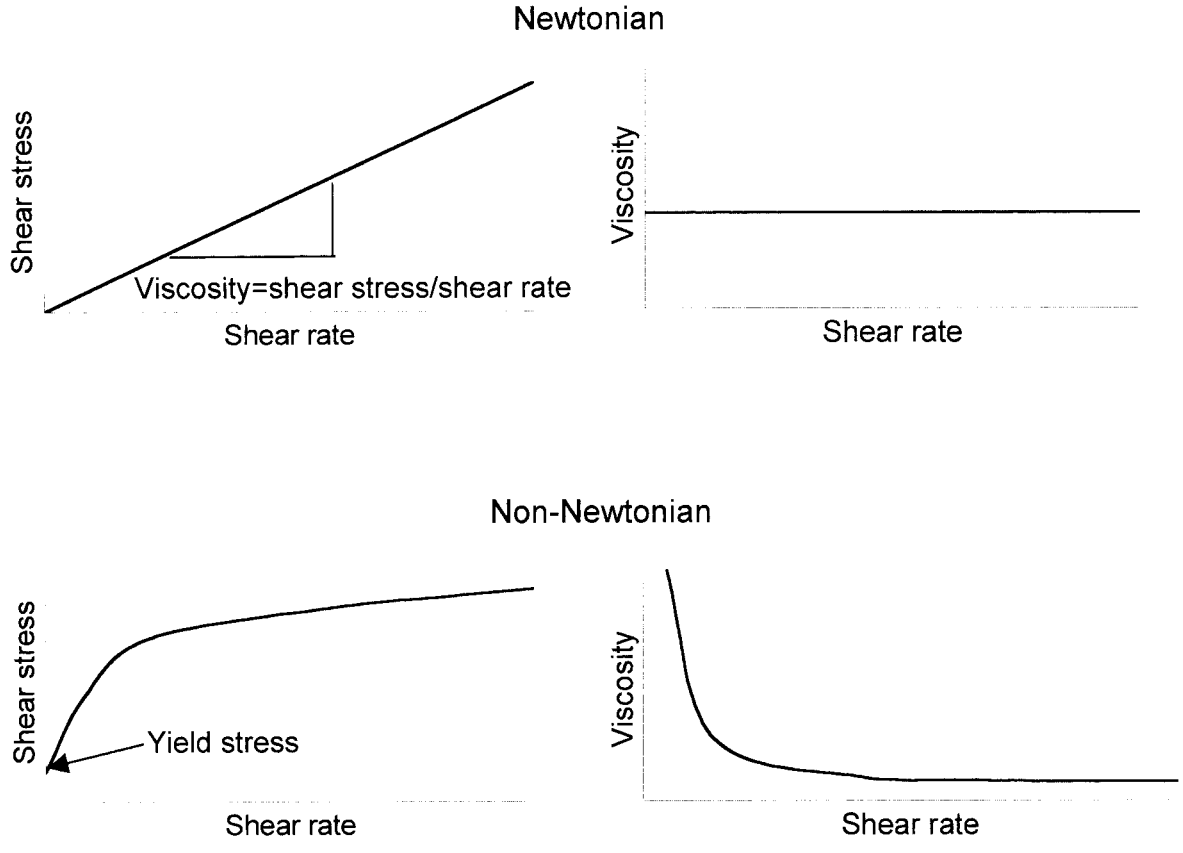

Figure 1 Shear stress–shear rate and viscosity–shear rate relations for Newtonian and non-Newtonian liquids.

shear stress examined, and thus the viscosity is constant. (2) In non-Newtonian liquids, the apparent viscosity is not a constant but rather depends on the magnitude of the shear stress or shear rate and can be calculated as the ratio of shear rate to shear stress. The apparent viscosity of a non-Newtonian fluid may decrease (shear-thinning behavior) or increase (shear-thickening behavior) as the shear rate is increased. Non-Newtonian liquids may have a yield stress below which there is a finite stress but the shear rate is zero (no flow), resulting in an infinite value for apparent viscosity.[14] The flow behavior of non-Newtonian liquids may also be time dependent; the viscosity of a thixotropic liquid decreases with time at a fixed shear rate.[10] Note that for both classes of fluids, the viscosity of a liquid depends on its temperature, and for most fluids viscosity decreases with increasing temperature. Several units have been used for viscosity, with the most common being millipascals.sec (mPa.sec), which is numerically equal to centipoise (cP); water at 20°C has a viscosity of 1.0 mPa.sec or 1.0 cP.

The viscosity of a liquid can be measured by a viscometer, which is a device built for studying stress-strain relations.[9,12] Capillary viscometers are the most widely used devices for measuring viscosity of Newtonian liquids. The working principle of a capillary viscometer is based on the measurement of flow rate of the liquid through a well-defined capillary tube under a certain pressure difference; at constant temperature and pressure difference, the flow rate decreases with increasing viscosity. Capillary viscometers can also be used for flow measurements of non-Newtonian liquids, but estimation of viscosity is difficult because the shear rate varies across the diameter of the tube (i.e., maximum at the wall, zero at the center). Rotational viscometers of various types are thus more commonly used for studying non-Newtonian liquids. In a rotational viscometer, the liquid under investigation is sheared between two surfaces, either under constant shear stress or shear rate, and the response (resulting shear rate or shear stress, respectively) is measured. The geometric design of the shearing portion varies among instruments but is usually designed to provide a uniform shear rate or shear stress throughout the sample being studied.

DEFINITION OF HEMORHEOLOGY

Hemorheology deals with the flow and deformation behavior of blood and its formed elements (i.e., RBCs, WBCs, platelets).[8] The rheological properties of blood are of basic science and clinical interest: the details of blood rheology are still being studied, and blood rheology can be altered in many disease states. There is an increasing amount of clinical and experimental data clearly indicating that the flow behavior of blood is a major determinant of proper tissue perfusion.

RHEOLOGY OF BLOOD

From a biological point of view, blood can be considered as a tissue comprising various types of cells (i.e., RBCs, WBCs, and platelets) and a liquid intercellular material (i.e., plasma). From a rheological point of view, blood can be thought of as a two-phase liquid; it can also be considered as a solid-liquid suspension, with the cellular elements being the solid phase. However, blood can also be considered as a liquid-liquid emulsion based on the liquid-like behavior of RBCs under shear.

Blood Viscosity, Ex Vivo

Because blood is a non-Newtonian suspension, its fluidity cannot be described by a single value of viscosity. Rotational viscometers allow the measurement of viscosity over a range of shear stresses (or shear rates), yielding a flow or viscosity curve for a blood sample.[9]

As shown in Figure 2, normal human blood exhibits shear-thinning behavior. At low shear rates or shear stresses the apparent viscosity is high, whereas the apparent viscosity decreases with increasing shear and approaches a minimum value under high shear forces.[9,15,16] At high shear rates above 100 to 200 sec^{-1}, the viscosity of normal blood measured at 37°C is about 4 to 5 cP and is relatively insensitive to further increases of shear. However, the viscosity becomes increasingly sensitive to shear rates below 100 sec^{-1} and increases exponentially as the shear rate is decreased. Nominal values for the viscosity of normal blood are approximately 10 cP at 10 sec^{-1}, 20 cP at 1 sec^{-1}, and 100 cP at 0.1 sec^{-1}.[16] Thus, at lower shear rates, blood viscosity becomes extremely sensitive to the decrement in shear forces. At stasis, normal blood has a yield stress of about 2 to 4 mPa.[9,14]

Determinants of Blood Fluidity

Because blood is a two-phase liquid, its fluidity at a given shear rate and temperature is determined by the rheological properties of the plasma and cellular phases and by the volume fraction (i.e., hematocrit) of the cellular phase.

PLASMA VISCOSITY

Plasma is the suspending phase for the cellular elements in blood, and thus a change in its viscosity directly affects blood viscosity regardless of the hematocrit and the properties of the cellular elements. The normal range of plasma viscosity is between 1.10 and 1.35 cP at 37°C,[12] but higher values are seen in disease states or after tissue injury. Plasma is a Newtonian fluid (i.e., viscosity independent of shear rate), yet technical artifacts have led some to report non-Newtonian behavior. In general, the level of plasma viscosity is a good, nonspecific indicator of disease processes and is increased in pathophysiological

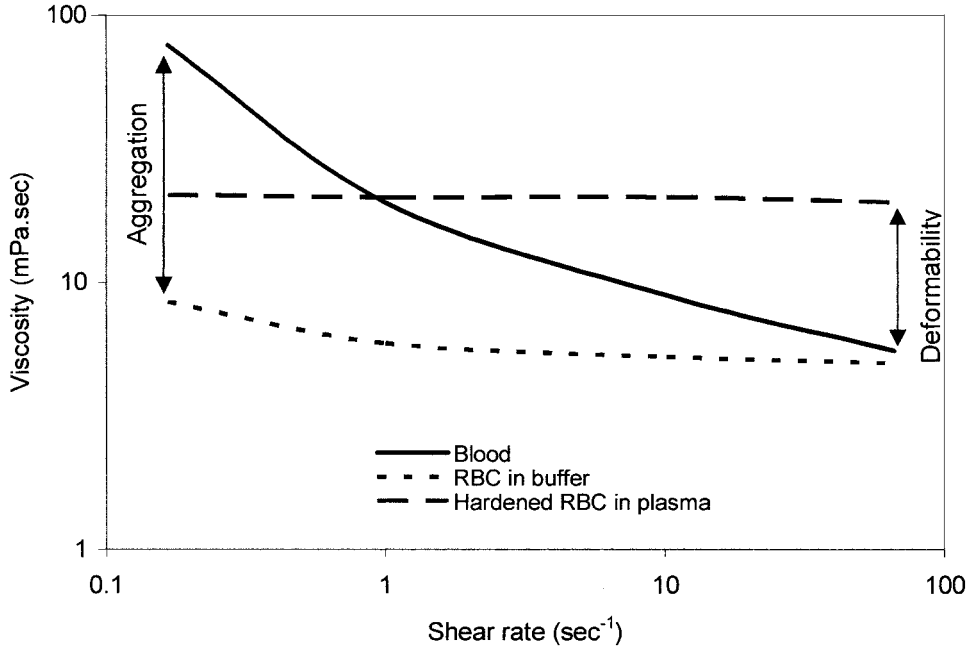

Figure 2 Shear rate–viscosity curves for normal blood, RBCs suspended in protein-free buffer (i.e., in a medium that does not induce RBC aggregation), and chemically rigidified RBCs suspended in plasma. The differences in viscosity at the lower and upper end of the shear rate range demonstrate the effects of RBC aggregation and deformability, respectively.

conditions associated with acute phase reactions.[17] This increase is closely related to the protein content of plasma. Acute phase reactants, such as fibrinogen, contribute significantly to the nonspecific increase of plasma viscosity in disease processes. Plasma viscosity can increase up to 5 to 6 cP in patients with abnormal protein levels such as seen in clinical states termed paraproteinemias.[18]

HEMATOCRIT VALUE

Under laminar flow conditions, the presence of cellular elements disturbing the flow streamlines is the primary reason why blood viscosity is higher than plasma viscosity is.[15] The contribution of this disturbance to the magnitude of blood viscosity can be appreciated by calculating the relative viscosity of blood (i.e., blood viscosity divided by plasma viscosity). With increasing amounts of cells, flow lines are progressively disturbed, and relative viscosity increases above its value of 1.0 for plasma alone. The degree of disturbance of flow streamlines, and consequently the viscosity of blood, thus strongly depends on the concentration of the cellular elements (i.e., hematocrit). As shown in Figure 3, there is an exponential relationship between the hematocrit value and blood viscosity, such that at higher levels of hematocrit, blood viscosity becomes increasingly sensitive to hematocrit alterations. At medium to high shear rates, there is about a 4% increase of blood viscosity per unit increase of hematocrit (e.g., a change from 45 to 46% hematocrit increases blood viscosity by 4%).[19]

CONTRIBUTION OF RED BLOOD CELL RHEOLOGICAL BEHAVIOR TO BLOOD FLUIDITY

In addition to the concentration of cellular elements in blood, their rheological properties are important determinants of blood fluidity. That is, the disturbance of flow streamlines depends not only on the concentration of blood cells but also on the behavior of these cells under shear forces (Fig. 4). RBCs are the major determinant of this effect, with these cells exhibiting a very special rheological behavior. Normal RBCs are highly deformable bodies and tend to orient themselves with the flow streamlines, especially if the shear forces are high enough to slightly deform these cells. In fact, it has been observed that RBCs behave like fluid drops under most flow conditions.[20] Thus, RBC deformation and orientation are the primary cellular factors affecting blood viscosity at high shear rates.[20,21]

Another important rheological feature of RBCs is their tendency to aggregate into linear arrays, termed rouleaux, in which they are arranged like stacks of coins. Linear aggregates then interact to form three-dimensional structures.[14] Fibrinogen and other large plasma proteins promote RBC aggregation, with aggregation dependent on the magnitude of shearing forces acting on the cells. Increased shear disrupts the aggregates, whereas reduced shear favors aggregation.[22] Because of the increased effective particle size, the disturbance of flow streamlines becomes more pronounced when RBC aggregates are formed and blood viscosity is significantly increased. RBC

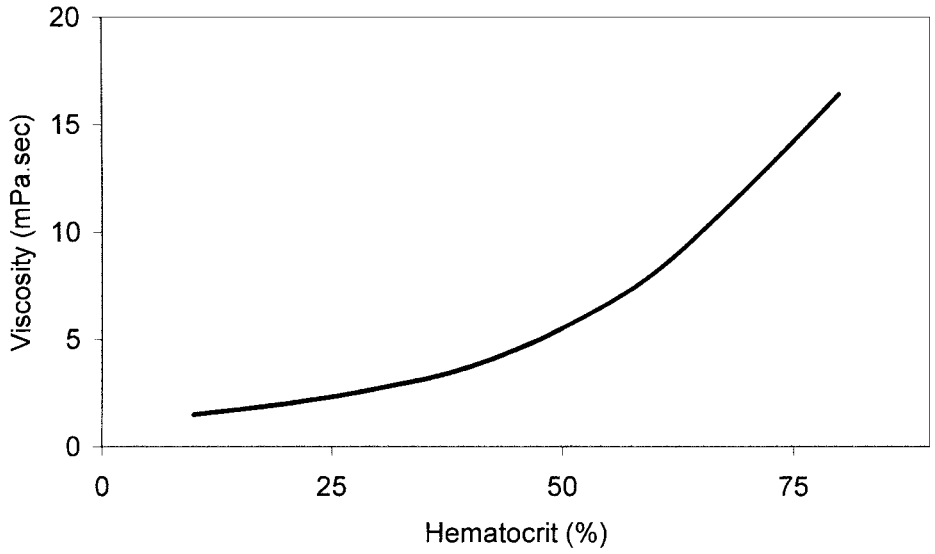

Figure 3 Effect of hematocrit on blood viscosity.

aggregation is thus the major determinant of blood viscosity under low shear conditions.

It is obvious from the previous discussion that when studied in large geometry systems (i.e., large blood vessels, rotational viscometers with large spaces between measuring surfaces), the non-Newtonian behavior of blood is closely related to RBC deformability and RBC aggregation.[15,23,24] RBC deformability and aggregation also affect blood flow in smaller blood vessels and in the microcirculation.[23,25,26] Blood cellular elements other than RBCs (e.g., various WBCs, platelets) have no significant effect on the macroscopic flow properties of blood (i.e., blood viscosity measured in large geometry systems) but may contribute markedly to blood flow resistance and flow dynamics in the microcirculation where vessel diameters are 100 μm or less.[27]

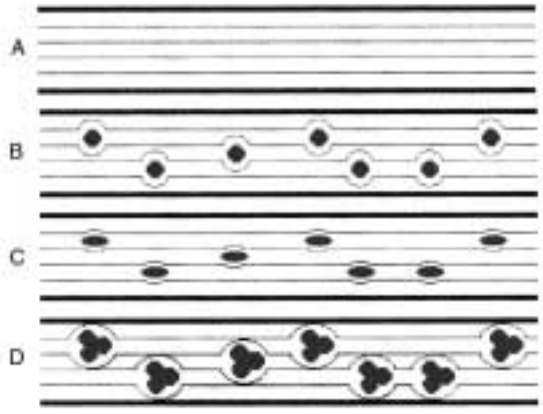

Figure 4 Effect of RBCs suspended in plasma on the flow streamlines. (A) Flow streamlines of plasma in the absence of RBCs, (B) distortion of streamlines in the presence of nondeforming RBCs, (C) decreased distortion of streamlines because of the deformability of RBCs, and (D) increased distortion due to RBC aggregation.

Red Blood Cell Deformability

RBCs are highly specialized cells that carry oxygen from the lungs to tissues and allow carbon dioxide to move from tissues to the lungs. Mature RBCs are biconcave disks about 8 μm in diameter and 2 μm thick. The unique shape and structure of RBCs confer special mechanical properties to these cells.[23,28,29] RBCs respond to applied forces by extensive changes of their shape, with the degree of deformation under a given force known as RBC deformability. The extent and geometry of these shape changes are functions of the magnitude and orientation of the applied forces, with RBC cellular properties as important determinants of the degree of deformation under a given stress. RBCs behave as elastic bodies, and thus the shape change is reversible when the deforming forces are removed.[30] RBCs also exhibit viscous behavior and thus respond as a viscoelastic body. Like shock absorbers on cars, the force needed to deform a RBC increases with both the extent and the rate of deformation. In addition, the RBC membrane can exhibit plastic changes under some pathological circumstances and can be permanently deformed by excessive shear forces.

The RBC membrane, including its underlying cytoskeleton, is the structured element that primarily determines the cell's dynamic mechanical behavior.[31] The lipid bilayer of the membrane is purely viscous and makes almost no contribution to the elastic behavior of the RBC membrane. Rather, it is now generally accepted that the RBC membrane cytoskeleton is mainly responsible for the maintenance of biconcave-discoid shape.[32] The RBC membrane cytoskeleton is a network of proteins lying just beneath the cell membrane, with the protein spectrin the most important component of this network.[33] The spectrin network is attached to the membrane integral proteins such as band 3 and glycophorins. Although the details of the network organization are not com-

pletely resolved, there is an increasing amount of data suggesting that the organization depends on maintaining RBC intracellular homeostasis. For example, membrane rigidity seems to depend on cytosolic calcium concentration, and thus the maintenance of normal mechanical behavior depends on a low cytosolic calcium level maintained by an active ATP-dependent calcium pump within the RBC membrane.[34]

In addition to membrane elastic and viscous properties as determinants of RBC deformability, two additional factors also contribute to this cellular property[29]: (1) the cytoplasmic viscosity of RBCs, which in normal RBCs is solely determined by the hemoglobin concentration, and (2) the biconcave discoid geometry, which provides excess area for the contained volume and thus enables shape changes without increasing the surface area of the membrane. It is obvious from geometric principles that an increase of surface area is necessary in order to deform a sphere, yet the RBC membrane is extremely resistant to area increases. The degree of hydration of the cell is thus an important determinant of its surface area–volume relationship: if RBCs are overhydrated, their volume will increase, whereas their surface area remains unchanged, thereby reducing cell deformability. Conversely, the cytosolic concentration of hemoglobin, and hence the cytosolic viscosity, is increased when cells are underhydrated, thereby also leading to reduced cell deformability. Active cation pumps control the intracellular volume of RBCs, maintaining both the special geometry and the normal cytoplasmic viscosity.[34]

RBC deformability can be assessed by monitoring the passage of RBCs through cylindrical pores with diameters smaller than the size of RBCs.[35] In general, the time needed to transit such pores at a constant pressure is determined, with longer times indicating reduced RBC deformability.[36] RBC deformability can also be quantitated by monitoring cell shape changes resulting from applied fluid forces, either by direct microscopic visualization or by analysis of laser-diffraction patterns generated by the deformed cells.[37]

Red Blood Cell Aggregation

If RBCs are suspended in autologous plasma and observed at rest via light microscopy, they form large aggregates resembling a stack of coins (Fig. 5). These aggregates, known as rouleaux, are easily dispersed by fluid forces (e.g., generating a local flow by applying a pressure on the coverslip) but rapidly form again when the fluid forces are removed.[14,22] However, such aggregation does not occur if RBCs are suspended in simple, isotonic salt solutions, and several studies have indicated that the extent and rate of RBC aggregation strongly depend on the type and concentration of macromolecules in the suspending medium.[14] In plasma, fibrous rather than globular proteins are responsible for aggregation, with fibrinogen concentration being the most important determinant of the aggregating property of plasma.[14] Other macromolecules, such as high-molecular-weight dextrans or other water-soluble polymers, can also induce RBC aggregation.[38] Although earlier studies suggested that the macromolecular composition of the suspending medium was the only determinant of RBC aggregation, more recent studies have shown that RBC cellular properties also play a very important role in the aggregation process.[38–44] The term aggregability has thus been used to express the intrinsic aggregation behavior of RBCs regardless of the properties of the suspending medium.[45]

Figure 5 RBC aggregates. (Reproduced from Schmid-Schönbein H, Grunau G, Brauer H. Exempla hämorheologica "Das strömende Organ Blut." Wiesbaden, Germany: Albert-Roussel Pharma GmbH; 1980)

The process of RBC aggregation can be considered the result of a balance between aggregating and disaggregating forces; disaggregating forces include fluid shear forces, electrostatic repulsion between cells, and the elastic energy of the cell membrane.[22,38,46] There are two coexisting yet mutually exclusive "models" for RBC aggregation: (1) Bridging Model, in which aggregation occurs when bridging forces due to adsorbed macromolecules on adjacent cell surfaces exceed disaggregation forces,[46–48] and (2) Depletion Model, in which a preferential exclusion of macromolecules from the RBC surface generates an osmotic gradient and fluid movement away from the intercellular gap and thus decreased cell-solvent affinity.[49–52] It is obvious from these two models that there is still disagreement regarding the exact nature of the aggregating forces. In particular, these two models predict contradictory effects on RBC aggregation with increased concentration of macromolecules near the cell surface. According to the bridging model, aggregation should increase, whereas the depletion model predicts decreased aggregation. Numerous attempts to

directly determine the extent of macromolecular adsorption on the RBC surface have been unsuccessful because of experimental artifacts,[14] thus preventing a clear distinction between the two models. However, recent RBC electrophoresis studies have provided important data suggesting that the local viscosity, and hence the local macromolecule concentration, is lower than the bulk phase, thereby supporting the depletion model.[51–54] In addition, a recent study that considered the magnitude of forces due to depletion and electrostatic repulsion has provided theoretical support for the depletion model.[52]

RBC aggregation can be assessed by several methods, of which the most widely used is the erythrocyte sedimentation rate (ESR) test, in which the sedimentation of RBCs in a vertical glass tube is observed. However, the ESR is a relatively slow method and provides only one type of data (i.e., RBC aggregate sedimentation after 1 hour). Newer automated methods based on photometric techniques have been developed; these devices are based on the measurement of light reflection from or light transmission through RBC suspensions. Aggregation reduces the number and increases the size of particles, and thus information related to the extent, rate, and strength of aggregation can be obtained.[55,56] Microscopic techniques can also be used to quantify RBC aggregation by observing the number of aggregates in a defined volume of a dilute RBC suspension.[14]

Contribution of White Blood Cells to Blood Flow at Tissue Level

WBCs have a negligible effect on whole-blood viscosity in large vessels because their number and volume concentration are small relative to the other cellular elements of blood. However, in the microcirculation, where blood vessel sizes approach or are even smaller than the size of blood cells, every single cell may have the potential to influence flow in the microvessel through which it is passing.[27,57]

The geometry and mechanical properties of WBCs depend on the type and status of the cell. Granulocytes (i.e., polymorphonuclear leukocytes) can undergo an activation process and exhibit extensive biochemical, morphological, and mechanical alterations as a result of activation.[58–60] The resistance encountered by a WBC while passing through the microcirculation therefore depends on its type and status and can be estimated to be several orders of magnitude greater than that for a RBC.[59] Consequently, their transit time through the microcirculation is much longer than that for RBCs, and they can transiently block certain channels of the microcirculation.[27] This blockage may be especially important in pathophysiological states (i.e., severe infection) in which the WBCs becomes activated and hence more rigid.

CLINICAL ASPECTS OF BLOOD RHEOLOGY

Hematocrit as a Determinant of Whole-Blood Viscosity

As discussed earlier, whole-blood viscosity is strongly dependent on hematocrit. Hematocrit in a given individual may not remain constant but rather is a dynamic parameter that may change rapidly and significantly as a part of physiological, pathophysiological, and even psychosomatic processes.[61] An acute rise in hematocrit might be the result of a relative increment of RBC mass in the circulatory system because of a reduction of intravascular volume. The primary cause of this volume reduction may be fluid loss by various means (e.g., gastrointestinal and urinary tracts, perspiration) or may result from constriction of the circulatory system that shifts the balance of forces governing the fluid exchange at the tissue level according to Starling's hypothesis. A well-known example of the latter situation is catecholamine discharge under acute stressful conditions, which results in a significant reduction of the volume of the circulatory system and a significant increase of blood pressure. A fluid shift from the vascular space to the interstitial area then follows this acute alteration, resulting in a higher hematocrit in the vasculature even if there is not an absolute increase of RBC mass. In addition, this fluid shift also affects the protein concentration of plasma, increasing plasma viscosity; RBC aggregation may also be increased as a result of increased fibrinogen concentration.

Stimuli such as catecholamine discharge may also acutely affect the absolute RBC mass actively circulating within the vascular system. Most mammals have a reserve volume of RBCs in the splanchnic region, and this volume can rapidly be introduced into the circulating bloodstream and contribute to the increased hematocrit during acute stress conditions. This "hematocrit reserve" is limited in humans but is well-developed in other species and actively used during exercise. Horses, for example, have a large splanchnic RBC reserve, and during strenuous exercise their hematocrit can be increased by more than 50% from the resting value.[62] Such rapid fluctuations in hematocrit and hence blood viscosity can often be compensated for by vascular autoregulatory mechanisms, in which the metabolic demands of the tissue promote dilation of blood vessels. However, such compensation can only occur if there is sufficient autoregulatory reserve within the tissue. If this reserve has already been depleted because of another hemodynamic stress (e.g., altered vascular geometry, lack of appropriate perfusion pressure), then the extra hemorheological load introduced by hematocrit increases can significantly and negatively affect tissue functions.[63]

The term stress polycythemia has been used to distinguish a chronic increment of RBC mass resulting from increased RBC production in bone marrow from

an acute increase in hematocrit because of the previously mentioned mechanisms.[61] Nevertheless, the hemodynamic results of increased hematocrit are the same regardless of the underlying mechanism. However, there is some uncertainty regarding the exact implications of hematocrit alteration in terms of its effect on oxygen transfer to tissues. On the one hand, the oxygen-carrying capacity of a given amount of blood is directly and linearly proportional to the hematocrit. Therefore, oxygen delivery to a tissue at a constant flow rate is higher if the hematocrit of the perfusing blood is higher. On the other hand, increased hematocrit results in a nonlinear increase of blood viscosity (see Fig. 3) and flow resistance, and thus the blood flow rate might be decreased, reducing the amount of blood perfusing a given tissue. This complex relationship thus leads to the concept of an optimum value of hematocrit at which the oxygen delivery to tissues is maximum.[64]

Pathological Alterations of RBC Mechanical Properties

Both RBC deformability and aggregation are rheological parameters that can be affected by pathophysiological processes. The normal rheological behavior of RBC is strongly dependent on the maintenance of an appropriate microenvironment and the preservation of metabolic functionality. Failure of these conditions can result in reversible or irreversible deterioration of RBC rheological behavior. Thus, both local and systemic disturbances of homeostasis have the potential to induce RBC rheological alterations.

EFFECTS ON RBC DEFORMABILITY

Maintenance of normal RBC deformability depends on the availability of metabolic energy in the form of adenosine triphosphate (ATP). ATP is required for the cation pumps in the RBC membrane (i.e., Na^+-K^+ATPase and Ca^{2+}ATPase) that serve to regulate intracellular cation and water content, thereby maintaining cell volume and thus the cell's surface to volume ratio.[34] The source of ATP within the cell is glycolysis, with about 90% of glycolysis in the RBC being anaerobic. Glucose supply to the RBC is critical for the maintenance of this mechanism because RBCs do not store glucose; their metabolism thus depends on the availability of glucose in their microenvironment. In addition to providing the metabolic energy for ATP, anaerobic and aerobic glycolysis are involved in metabolic pathways for several cofactors of the antioxidant defense system. Although under most physiological conditions, glucose availability is not a limiting factor, RBC geometric and mechanical alterations can be detected in blood stored for prolonged periods of time.[65] Such changes because of metabolic depletion may also occur in RBCs trapped in ischemic tissues for prolonged periods; decreased pH in ischemic tissues may also affect RBC deformability.[66]

In addition to the fluid-electrolyte balance of the RBC, and hence its volume and cytoplasmic viscosity, the mechanical properties of the cell membrane are major determinants of its deformability.[28–32] It has been reported by various groups that alterations in the lipid composition of the RBC membrane have only minor effects on mechanical behavior, whereas alterations in membrane skeletal proteins play a major role.[28–32] It is well-known that hereditary abnormalities in major membrane skeletal proteins are associated with shape changes and impaired deformability in RBCs.[67] Similar alterations can also be observed in various pathophysiological processes, primarily as a result of abnormal associations among the normal components of the RBC membrane cytoskeleton.

Increased cytosolic calcium concentration is a frequently detected alteration associated with reduced RBC deformability.[68,69] Relations between cytosolic calcium concentration and mechanical alterations have been reported in peripheral vascular diseases and exercise and as a result of drug therapy or hormone level abnormality.[70–74] It has been experimentally demonstrated that an increased calcium level rigidifies the cytoskeletal network, most likely through a calmodulin-dependent mechanism.[75,76] The exact site of this interaction is not clear, but spectrin-actin band 4.1 binding sites are considered to be involved.[77] The effects of such calmodulin-calcium complexes on associations in the cytoskeletal network seem to be reversible on lowering of the cytosolic calcium concentration. Chemical reactions that increase cross-linkages among membrane skeletal proteins also rigidify the RBC membrane and reduce cell deformability; oxidative alterations in RBC induce such cross-linking among membrane proteins and play a significant role in the mechanical deterioration of RBCs.[78,79]

EFFECTS ON RBC AGGREGATION

Increased RBC aggregation is a well-known consequence of acute tissue injury such as myocardial infarction, inflammation, or trauma; increased plasma levels of a group of proteins known as acute phase reactants are responsible for this increase.[17] Fibrinogen is the most important acute phase reactant; others include C-reactive protein, serum amyloid A, haptoglobin, and ceruloplasmin.[17] Recent studies have indicated that RBC surface properties that affect aggregation are also altered in pathophysiological states.[41–43,51,66,80] These changes of surface properties have been demonstrated by the increase of aggregation observed for RBCs suspended in a standard aggregating medium; increased RBC "aggregability" has been noted in sepsis and after ischemia–reperfusion injury.[43,51,56] This increased tendency for RBC aggregation is most likely related to decreased surface charge density and a shift of the balance toward aggregating forces because of decreased electrostatic repulsion among adjacent RBC.

Recent RBC electrophoresis studies of cells in various high-molecular-weight dextran solutions have

suggested that alterations of other surface properties may also play a role in modified aggregability.[51,52,81,82] Evaluation of experimental data for RBCs exposed to oxidant stress and to activated WBCs indicates that the depletion layer near the RBC membrane is decreased, most likely because of structural changes of the RBC glycocalyx.[41,42]

ROLE OF OXIDANT STRESS IN HEMORHEOLOGICAL DISTURBANCES

Oxygen-free radicals are generated in biological systems during various physiological and pathophysiological processes and are important elements of cellular metabolism and the defense systems of higher organisms. However, there is also a negative aspect of oxygen-free radicals and related chemical species; they are strongly toxic to the organism because they can attack and oxidatively modify a wide variety of biological molecules. Oxygen-free radicals are involved in ischemia–reperfusion injury, in which activated leukocytes generate these reactive species.[83,84] In such pathophysiological states, tissues and cells that are exposed to these exogenous oxygen-free radicals can be damaged, with RBCs among the most susceptible cells. RBCs are also affected by free radicals generated within the red cell itself. In the circulation, RBCs are exposed to high oxygen concentrations and are also rich in iron, a transition metal that promotes the formation of oxygen-free radicals.[85] Under normal conditions, free radicals are continually generated in the highly catalytic medium of the RBC, yet well-developed antioxidant defense mechanisms usually prevent their deleterious effects.[85] However, if the generation of oxygen-free radicals exceeds the capacity of the defense mechanisms, several structural and functional modifications occur in the RBC. These modifications include formation of methemoglobin, increased lipid peroxidation, oxidative modifications and degradation of proteins, cross-linking between membrane cytoskeletal proteins, attachment of hemoglobin to membrane cytoskeletal proteins (mostly to spectrin), altered passive cation permeability, and altered surface properties.[41,79,86–88]

Recent evidence indicates that the effects of oxygen-free radicals on RBC properties depend on their site of generation (i.e., extracellular or intracellular) as well as on the concentration of these radicals.[41] Experimental studies using the xanthine oxidase–hypoxanthine system to generate superoxide anions outside of the RBC indicate that RBC aggregability, rather than deformability, is primarily affected. Aggregation was increased at lower concentrations and inhibited at higher levels. In contrast, experiments with agents that generate superoxide anions inside the RBC by reacting with cytoplasmic hydrogen donors cause deterioration of RBC deformability, while having only a very slight effect on RBC aggregation.[41]

ROLE OF WBC ACTIVATION IN HEMORHEOLOGICAL DISTURBANCES

Activation of polymorphonuclear leukocytes (PMN) is a major aspect of inflammation and is thus encountered by the organism during the course of various pathophysiological states.[89] In addition to increased rigidity, PMN activation is associated with an increased level of secretory activity, resulting in a massive production and release of chemotactic agents, oxygen-free radicals, and proteolytic enzymes by the cell.[58,59,89,90] These substances released by activated PMN can affect neighboring cells and tissues (e.g., vascular endothelial cells) as well as areas more distant to the release site and are known to be involved in the inflammatory response.

Activated PMN may also affect other blood cells, and it has been reported that activated leukocytes induce several structural and functional changes in neighboring RBCs.[42,79,91] These alterations include increased membrane lipid peroxidation and cell lysis and changes of RBC membrane cytoskeletal proteins (e.g., cross-linking between spectrin and hemoglobin) that are associated with decreased RBC deformability. Experimental studies also indicate increased aggregability of RBC incubated with activated PMN; these changes of aggregability are associated with altered RBC surface properties.[42] The effects of activated PMN were found to be minimized by both antioxidant enzymes and inhibitors of proteolytic enzymes, indicating that both oxygen-free radicals and proteolytic enzymes play a role in activated PMN-RBC interactions.[42,79]

ROLE OF HEMORHEOLOGY IN HEMODYNAMICS

There are extensive data in the literature indicating hemorheological alterations in a wide range of physiological and pathophysiological conditions. However, because almost all of these reports involve laboratory studies of rheological parameters (e.g., viscosity, aggregation, deformability), there is still uncertainty regarding the exact implications of these alterations for in vivo flow conditions and for tissue perfusion. This uncertainty is, in part, based on reported differences between the apparent viscosity of blood measured using a vascular bed as a "viscometer" and that measured with a tube or rotational viscometer. Thus it has been suggested that the in vivo influences of altered hemorheological factors might be different from those predicted based on measurements performed on blood samples outside the circulatory system. Therefore, a better understanding of hemorheology-hemodynamics relations requires studies of pressure-flow relations in flow systems that more closely approximate the mammalian vascular system.

Flow Behavior of Blood in Cylindrical Tubes

Cylindrical glass tubes are frequently used as highly simplified models of the vascular system; such tubes allow measuring pressure-flow relations of blood and also permit visualization of blood during flow.[92] Studies during the early 20th century indicated that the apparent viscosity of blood flowing through capillary tubes becomes lower as the tube diameter becomes smaller (the so-called Fahraeus-Lindqvist effect) reaches a minimum value around 6 to 8 μm and then increases sharply as the diameter becomes even smaller.[7] This "anomalous" behavior of blood is affected by RBC deformability and is less marked or even absent for suspensions of rigidified RBC.

Studies in the early 20th century also reported that RBCs are not evenly distributed throughout the cross-section of a cylindrical tube during flow but rather tend to accumulate in the central region, leaving a cell-poor layer close to the tube wall. Such a distribution obviously results in hematocrit levels that are maximum at the central zone and minimum at the peripheral zone.[93] It should be noted that the central fluid layers with minimum shear forces have the highest velocity, whereas those near the wall have the lowest. Therefore, the nonuniform distribution of RBC yields a higher average relative velocity for RBC compared with cell-poor, plasma-rich layers near the wall. As a result, the hematocrit of blood contained in a tube in which a radial hematocrit gradient exists is lower than the hematocrit of blood collected from the outflow, discharge end of the tube (the so-called Fahraeus effect).[7,94] Because blood viscosity is a function of hematocrit (see Fig. 3) and because the hematocrit in the tube is reduced, the viscosity of the blood within the tube is lower than that predicted based on the discharge hematocrit. RBC deformability affects this reduction of hematocrit in small tubes such that it is less marked for suspensions of rigid RBC.[7]

The axial migration and radial distribution of RBC mentioned earlier are expected to reduce the flow resistance in a tube by reducing the suspension viscosity at the tube wall.[92] The frictional resistance at the vessel wall is directly proportional to the fluid viscosity at this position and is a significant component of the hydrodynamic resistance. RBC aggregation and sedimentation also affect RBC distribution across the diameter of the tube, with the effects on flow resistance dependent on the orientation of the flow system with respect to gravity[92,95–97]: (1) sedimentation of RBCs tends to increase the flow resistance in horizontal tubes because RBCs accumulate on the lower side of the tube wall and (2) in vertical tubes axial migration of RBCs dominates and flow resistance is reduced because of the formation of a cell-poor, lower viscosity region near the tube wall. Several groups have demonstrated that in vertical tubes increased RBC aggregation promotes the formation of a cell-poor layer near the wall and thus a reduction of flow resistance. Blood flow in cylindrical tubes is thus at least a function of RBC aggregation, RBC deformability, tube diameter, and orientation versus gravity.[92,95–97] Given these considerations, it is not unexpected that the behavior of blood in tissue might be significantly different than that predicted based on measurements in large-scale viscometers.

Flow Behavior of Blood In Vivo

Several factors suggest that experimental results in glass tubes may not be directly applicable to in vivo flow conditions. (1) The geometry of the microvasculature is extremely complex, with frequent branching, and thus the residence time of blood within a single straight vessel may be too short to allow development of a cell-poor zone. (2) The orientation versus gravity of the individual microvessels varies throughout the body, and thus hydrodynamic effects related to the development of cell-poor zones in a given vessel depend critically on its orientation. (3) Vascular control responses may counter or modulate effects based on rheological findings in rigid glass tubes, inasmuch as the vascular system is equipped with a very effective control mechanism for adjusting the geometric component of hydrodynamic resistance to match blood flow to tissue metabolic needs. Further, blood vessels have elastic walls, and their diameters can increase considerably with increased blood pressure, thereby modifying vascular geometry and flow resistance. In fact, active and passive geometric changes of the vascular system represent one of the main challenges to the prediction of in vivo blood flow based on laboratory hemorheological data.

ROLE OF RBC DEFORMABILITY

RBC deformability has significant effects on flow resistance in all areas of the vascular system.[23] In large blood vessels, deformable RBCs are easily oriented in the flow streamlines and thereby reduce blood viscosity; impaired RBC deformability limits cell orientation in flow and thus increases blood viscosity.[15] In smaller blood vessels, the dependence of the Fahraeus-Lindqvist effect on RBC deformability affects the deformability-related reduction in flow resistance.[7,94,98,99] In addition, as the vessel size becomes less than several hundred microns, axial migration and related phase separation become important mechanisms that affect flow resistance; axial migration is promoted by RBC deformability and reduced as the cell becomes rigid.[7,98] In the true capillaries, where the RBC must deform to enter and transit vessels smaller than the resting cell diameter, RBC deformability is the most important factor affecting the flow of blood (Fig. 6).[100,101]

ROLE OF PHASE SEPARATION AND RBC AGGREGATION

Phase separation, axial migration, and the formation of a marginal cell-poor fluid zone near the vessel wall are also features of in vivo blood flow.[102] In addition to af-

Figure 6 RBCs need to alter their shape extensively in order to be able to pass through microcirculation. (Reproduced from Schmid-Schönbein H, Grunau G, Brauer H. Exempla hämorheologica "Das strömende Organ Blut." Wiesbaden, Germany: Albert-Roussel Pharma GmbH; 1980)

fecting flow resistance, these phenomena can lead to an alteration of the average hematocrit of blood in branching blood vessels.[103–107] As illustrated in Figure 7, side branches of blood vessels are fed by the marginal stream that has a reduced hematocrit, thereby resulting in lower hematocrit vis-à-vis the hematocrit in large blood vessels. Therefore, the average hematocrit of the blood in vessels of all sizes in a tissue, termed tissue hematocrit,[103,106,107] is lower than the hematocrit value measured in the blood obtained from a large vein or artery.

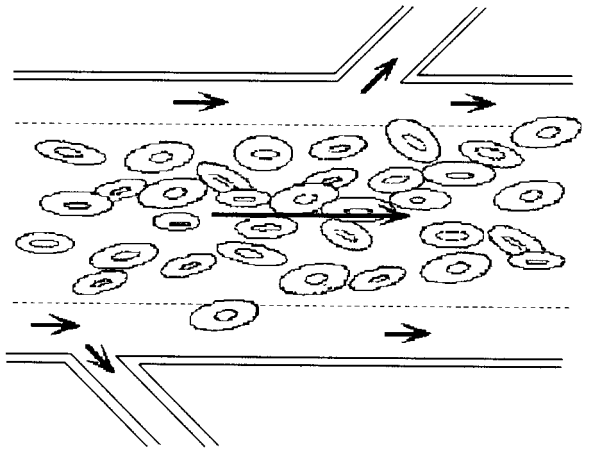

Figure 7 Accumulation of RBCs in the central zone of blood vessels during flow; side branches are fed by the cell-poor marginal fluid layer, resulting in a reduced tissue hematocrit.

Tissue hematocrit can be as low as one half of venous hematocrit in some tissues and is influenced by RBC rheological properties.

RBC aggregation is also an important factor in determining the degree of phase separation and related hydrodynamic effects,[95,96,108,109] yet its effects on in vivo flow resistance have not been fully resolved; predictions based on tube studies suggest increased flow resistance in large vessels and decreased resistance in smaller vessels. Furthermore, as the vessel becomes even smaller, RBC aggregates must be dispersed because of geometric considerations; disaggregation has an energy cost, and this adds to the hydrodynamic resistance.[110]

The several possible effects of RBC aggregation seem to be reflected in the findings reported by several investigators. In studies employing direct microscopy of small vessels, intensified RBC aggregation increases microvascular flow resistance.[111–113] Conversely, in whole-organ preparations in which pressure-flow relations have been investigated, several groups report that enhanced RBC aggregation decreased, increased, or had no effect on flow resistance.[114–118] In these whole-organ studies, the effects of RBC aggregation were found to depend on the intensity of aggregation as modulated by the concentration of aggregating macromolecules.[114] A moderate increase in RBC aggregation decreased flow resistance whereas greater aggregation resulted in increased resistance. It has thus been suggested that the controversy between direct microscopy and whole-organ stud-

ies can be resolved by considering the higher energy cost for disaggregation at the entrance of capillaries; this energy cost would be reflected in direct microscope studies but could be of minimal importance if a larger segment of the vasculature is considered.[110]

ROLE OF VASCULAR CONTROL MECHANISMS

Blood flow to a tissue is mainly controlled by vascular geometry (i.e., diameter), with vessel diameter determined by the state of contraction of smooth muscle in the vessel wall. Control of blood flow is directly related to the metabolic conditions of the tissue and normally functions to match the metabolic demands of the tissue with the blood flow supply to the tissue. Therefore, altering the vascular geometry component of flow resistance could compensate for any reduction in tissue perfusion resulting from adverse hemorheological alterations.

Nitric oxide (NO) is an important mediator of vascular control mechanisms.[119] It is synthesized by endothelial cells and dilates blood vessels by reducing the degree of smooth muscle contraction. NO synthesis by endothelial cells is affected by shear forces acting on these cells,[120,121] and thus any factor affecting the shear forces near the vessel wall should be expected to influence the geometric component of flow resistance.[122] For example, if wall shear stress is reduced as a result of enhanced RBC aggregation (i.e., increased axial migration), NO synthesis might be diminished, thereby leading to increased vascular tone. Therefore, vascular regulatory mechanisms need to be considered when evaluating the effects of hemorheological factors on in vivo blood flow.

Importance of Hemorheological Factors for Tissue Perfusion

Proper tissue metabolism and function are highly dependent on adequate blood supply, and most tissues are well-equipped with vascular control mechanisms that keep the blood supply and the metabolic demand of the tissue in balance. It is obvious from Poiseuille's equation that vascular flow resistance is a function of geometric factors, often termed "vascular hindrance" and viscosity-related factors. Although the vascular component has been recognized and studied for many decades, the importance of the rheological properties of blood as determinants of vascular flow resistance has only recently been appreciated.

Developments during the last 30 years have improved our understanding of blood rheology and tissue perfusion under dynamic conditions. It can now be postulated that impairments of blood rheological parameters, including the mechanical properties of RBCs and WBCs, should result in impaired tissue perfusion. However, the relative importance of hemorheological factors in pathophysiological processes is still unclear because of, at least, the following:

1. The interactions between blood rheological factors and hemodynamics are highly complex. The dynamic nature of vascular hindrance (i.e., blood vessel diameter) makes a significant contribution to this complexity, in that vascular regulatory mechanisms are always attempting to match tissue blood flow with the metabolic needs of the tissue. Thus, for normal tissue in which the vasculature has sufficient regulatory ability, rheological alterations may be compensated for by an appropriate change of vascular geometry. However, if the vasculature is disturbed by disease processes (e.g., arteriosclerosis), vascular regulatory mechanisms may not be sufficient to compensate for changes of blood rheology.
2. Blood rheological factors (e.g., plasma viscosity, RBC deformability, RBC aggregation, WBC activation) are sensitive to the metabolic status of the tissue being perfused. Any changes within the tissue, such as ischemia or infection, could readily affect the rheological properties of the various cellular elements in blood and thus alter one or more aspects of its rheological behavior. Thus there often is difficulty in determining cause and effect relations in pathophysiological states. Blood rheological alterations might be either the cause or the result of a pathophysiological process, and it may not be easy to distinguish between these two possibilities. In order to resolve this "chicken versus egg" dilemma, investigators are now studying blood rheological factors during and after therapy designed to correct a pathophysiological condition (e.g., hypertension, diabetes mellitus). Satisfactory completion of these studies and ongoing hemorheological investigations of several clinical disorders are expected to provide new and important information relevant to the role of blood rheological factors in health and disease.

ACKNOWLEDGMENTS

Preparation of this manuscript was supported, in part, by NIH Research Awards HL15722, HL48484, HL70595, and TW01295 and by Akdeniz University Scientific Research Projects Unit.

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