PROGRESS IN HEMATOLOGY

VOLUME VII

Edited by

Elmer B. Brown, M.D.

and

Carl V. Moore, M.D.

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With 23 Contributors

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Introduction

In hematology, as in other medical and scientific disciplines, the critical review serves an increasingly important function as research and its associated literature expand information at a rate that continues to accelerate. Students in training, teachers, practitioners, and investigators all feel the need for expert guidance to help identify, evaluate, and place in perspective the most significant contributions. So many different mechanisms have been developed for providing reviews, however, that each must be sure it serves a unique need and function. The editors of PROGRESS IN HEMATOLOGY attempt to meet this requirement by selecting topics that span the broad range of the discipline and are ripe for this kind of informational synthesis. They have invited authorities from the United States and abroad to review their areas of special competence in a selective, discriminating fashion to provide the guidance needed to stay abreast of the rapid changes in hematology.

Several of the reviews summarize newly acquired practical information of current importance in the management of patients or in the operation of a modern diagnostic laboratory. Others are at the leading edge of hematology where the interaction of traditional tools and concepts gives way to those of more basic disciplines. These forays into basic science require sustained concentration for those whose training has not provided the new vocabulary; the immediate usefulness of this material to the clinician may be obscure, but the pace of advances in hematology during the past two decades has, to a great extent, been due to the interjection of similarly new concepts. As in the past, practical application will be made by those whose minds are prepared to recognize the clinical correlations.

The editors express their great appreciation to the workers who accepted invitations and have contributed to Volume VII.

Elmer B. Brown
Carl V. Moore

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The Contribution of Normal and Pathologic Erythrocytes to Blood Rheology

P. L. LA CELLE and R. I. WEED

INTRODUCTION

Poiseuille, a French physician having an interest in flow characteristics of blood, performed careful in vitro and in vivo experiments of fundamental importance to fluid mechanics in general and to the development of blood rheology in particular. His early experiments with in vitro capillary systems were frustrated by thrombus formation. He then studied instead simple liquids and derived an expression predicting the flow behavior of simple fluids in cylindrical capillary tubes.[98] Subsequently, most investigators have been physical scientists concerned with fluid mechanics of blood. In the past decade an increasing appreciation of the significance of the properties of blood as a circulating fluid has developed, and considerable investigative effort has been directed toward elucidation of the contribution of abnormal blood components to pathophysiology of various diseases. Important new information has accumulated, particularly as the result of joint efforts of physical scientists and clinical investigators; however, interpretation and practical clinical application have not always kept pace with the concepts derived from clinical investigation. The purpose of this review is to outline some current concepts of rheologic features of blood as they apply to various portions of the circulatory system, to emphasize the contribution of erythrocytes to flow properties of blood, and to describe factors which affect erythrocyte rheologic properties. In addition, a critical analysis of the significance of altered blood rheology to various disorders will be presented, with some practical suggestions for therapy based on an appreciation of rheologic considerations.

CONCEPTS OF HEMORHEOLOGY

Rheology: Fluid Mechanics of Non-Newtonian and Viscoelastic Materials

Rheology, that branch of fluid mechanics dealing chiefly with non-Newtonian and viscoelastic substances, is concerned with deformation and flow properties. Blood, a complex liquid that behaves as a non-Newtonian fluid in its flow characteristics, has become the object of increasing research effort with the result that considerable literature has accumulated. Articles by Whitmore,[153,154] Merrill,[90] Johnson,[73] Burton,[14] Reiner,[108] and Wayland[137] provide insight into some basic biophysical concepts of rheology; for utility's sake, however, some definitions pertinent to

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rheologic systems and a description of some specific rheologic characteristics of the vascular system are presented here.

Newton observed that the resistance or internal friction between adjacent layers (laminae) of a flowing liquid sliding past each other is proportional to the area of contact between the layers and the velocity gradient, the rate of change of velocity in the direction of force application with respect to distance normal to the plane of shear (plane of sliding contact between layers). He formulated the equation relating flow and viscosity of a fluid:

$$F = A \cdot \eta \cdot \frac{\Delta V}{\Delta X}$$

where F, the shear stress, is the force imposed per unit area of fluid layer (dimension: dyne/cm^2), and where $\frac{\Delta V}{\Delta X}$, shear rate, is the velocity gradient. Shear rate, simply defined, equals the difference in velocity between the fluid layers divided by the distance between them (dimension: $\frac{\text{cm/sec}}{\text{cm}} = \text{sec}^{-1}$). A is the area of contact between the fluid layers. Therefore, η, the coefficient of viscosity, equals $F/A/\frac{\Delta V}{\Delta X}$, and viscosity is defined as the ratio of shear stress to strain rate.

The coefficient of viscosity has the dimension: poise = (dynes · sec) /cm^2. Water at 20°C has a viscosity of 0.01 poise; thus, the practical unit of viscosity is the centipoise (= 0.01 poise).

In all simple fluids, viscosity is independent of shear stress and velocity gradient or shear rate. However, in non-Newtonian liquids such independence is not observed at all values of shear stress and shear rate. Blood viscosity is dependent on shear rate particularly at rates less than about 50 sec^{-1}.[27, 28, 30, 89, 116] Blood also exhibits a yield stress; that is, any finite force below a certain threshold will cause elastic deformation without flow, and only above the threshold will flow be initiated. This contrasts with a simple fluid where any tangential force causes bulk flow. The yield stress may stem from the small attractive force normally existing between cells,[154] but more particularly from the three-dimensional network of protein (hemoglobin and non-hemoglobin) of the cell membrane.[35, 123, 141] The existence of a yield stress indicates that viscosity approaches infinity as strain rate approaches zero, since the shear stress may have a finite value under zero flow conditions. Conceptually, this is misleading;[87] however, in specific in vivo situations increased yield stresses are encountered, with practical significance to the relative viscosity of blood.

Elasticity implies, in simple mechanical terms, that applied force causes a reversible change of shape, or strain, of a material as long as the strain does not exceed a finite limit (elastic limit or yield point). Beyond the yield point irreversible distortion or deformation may occur. In blood it is considered that yield stress reflects disruption of a three-dimensional structure within the cell membrane,[90] disruption of rouleaux formation (cell-cell interaction), and, in some cases, alteration of relationships within the cell contents. In elastic materials, the permanent shape change or plastic deformation which occurs if an elastic limit is reached is dependent on the magnitude of the deforming force but is time independent. In viscoelastic substances such as the erythrocyte membrane,[74, 106, 107] recovery occurs if force application is brief, but flow of the substance results from prolonged stresses.

Characteristics of Blood Flow in Various Regions of the Circulatory System

General Characteristics. Under conditions of steady laminar (Poiseuille) flow of a simple liquid in a cylindrical tube of uniform diameter, a velocity profile exists across the tube such that the lamina at the tube axis has maximum velocity and the velocity decreases in a parabolic manner to a minimum value at the wall. The observations of Poiseuille[98] and later Copley and Staple[32] of an immobile plasma layer in blood in the wall region of small vessels, and the demonstration by Goldsmith[58-60] and others of a velocity profile with maximal axial velocity indicate similar behavior of this non-Newtonian fluid. Thus, the shear rate of a simple fluid is highest at the wall surface and lowest at the tube axis, and can be calculated as $4\bar{V}/r$ where $\bar{V}$ equals mean velocity of flow across the tube cross-section, and r equals the tube radius. Therefore, shear rates may be expected to vary greatly from one region of the circulation to another, and viscosity, dependent on the relationships of erythrocytes, plasma proteins, as well as on the shear stress and strain rate, may also be expected to differ. Wells and Merrill[146] have reported shear rates in arterioles to be relatively low, in the range of 5 to 25 sec^{-1}, and the in vivo data derived by Skovborg, Nielsen, and Schlichtkrull[127] from a rabbit ear perfusion technique gave the best correlations with cone-plate viscometric data obtained at shear rates 5 to 75 sec^{-1}, a range similar to that of Wells and Merrill. Fitz-Gerald[50] analyzed capillary flow and found greater resistance to flow than was predicted from the assumption of pure laminar flow. He observed that the dependence of resistance to flow in capillaries 5 to 7 μ in diameter is not directly related to velocity (i.e., erythrocyte velocity falls off very rapidly as pressure is reduced), emphasizing that viscosity may be high under conditions of capillary flow. Thus, the rheologic properties of blood would be expected to have the greatest significance in arterioles, capillaries, and postcapillary sinuses or venules where the viscosity may be high at the low shear rates encountered. Alterations of the properties of cells and plasma proteins which serve as "lubricants" for erythrocyte flow in narrow channels may cause marked changes in the rheologic properties of these regions.

Whitmore's[154] estimates suggest relatively high shear rates in the arterioles and capillaries as well as low viscosity. Prothero[101] observed low viscosity for rabbit blood flowing in a 5μ capillary under perfusion pressure of 600 to 1000 mm of mercury which would tend to support such estimates. It is difficult to reconcile the divergent conclusions, except to note that the high perfusion pressures are not physiologic, and the net flow may have exceeded the 0 to 1 mm/sec flow of capillaries in vivo. If such were the case, shear rates would have been high in this in vitro system and viscosity low. In studies with the micropipette system in which pressure to force normal erythrocytes through calibrated glass capillaries can be measured, very small forces were required if the capillary were greater than 3μ; large forces were needed when the channel was small. These observations imply low viscosity if the channel is not limiting and demonstrate the major role of the erythrocyte as the determinant of flow in small capillaries.[77, 78]

Chien's[30] suggestion that the ratio $4\bar{V}/r$ may be applied as an approximation of shear rate would yield the lowest shear rates in venules and small veins.

Blood viscosity is shear dependent as indicated in Figure 1 where whole blood viscosity is recorded as a function of shear rate in a cone plate viscometer at 37°C. Below 50 sec^{-1}, viscosity increases with decreasing shear rates, and, as the rate

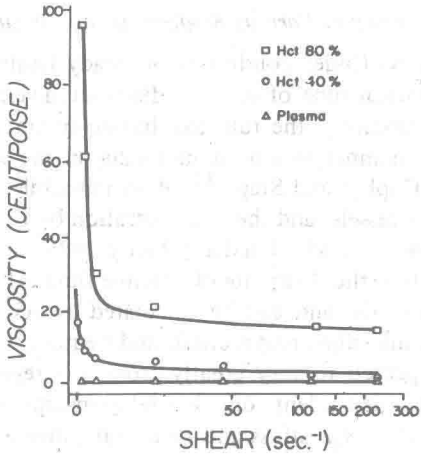

FIG. 1.–The relationship of whole blood viscosity to shear rate. Viscosity was measured in a cone-plate viscometer at 37°C at hematocrits of 40 and 80 percent. Viscosity of plasma is shown for comparison.

approaches zero, viscosity rises almost exponentially. Thus, in the circulation, the viscosity has been postulated to be highest in the venules and small veins, and lowest in the capillaries, where shear rates have been assumed to be low and high, respectively, assuming the in vivo behavior parallels that observed in the viscometer. The relative viscosity of blood, *if* Whitmore's estimates of shear rates were valid, would be similar throughout the circulatory system, assuming that in each circumstance blood behaves as a homogeneous fluid. Burton[14] and Whitmore[154] suggest an approximately constant viscosity could be assumed for blood: the value of η, as compared to water is suggested to be between 3 and 4 centipoise, with some cyclic variation in arterial vessels having pulsatile flow. This suggestion must be modified for microcirculatory regions where channels are less than 3 to 5μ and in the slowly flowing portions of the venous system, as well as for situations of pathologic reduction of flow rate; in these regions laws governing laminar flow are not valid.[153]

Large arterial vessels. The viscosity of blood depends in large part on the aggregation[27, 29, 35, 41, 89, 116] (rouleaux formation) and the deformability of the erythrocytes.[27, 117, 141, 150] The decrease in viscosity (shear thinning) at high shear rates results from disaggregation of rouleaux and shear deformation of the erythrocytes.[27, 35, 141, 142, 150] The axes of deformed erythrocytes align with the flow direction, decreasing viscous resistance.[60, 117] Decrease in viscosity at relatively low shear rates in large vessels also may result if rouleaux of sufficient length and number form so as to enhance axial rouleaux alignment and flow of cells as an axial plug with a cell-free layer at the vessel wall.[27, 49] For normal blood, rouleaux are dispersed and shear deformation is maximum at shear rates above 200 sec^{-1},[90, 116, 147] and viscosity becomes independent of shear rate.

In large vessels turbulence alters the idealized concept of laminar flow of blood; that is, laminae are disrupted at points of high rates of change of velocity (e.g. in regions of valves, change in vessel diameter, and at branch points). Burton[14] suggests that nonlinear "microturbulence" occurs at a microscopic level with disruption of laminar flow. Such anomalies would alter viscosity. Burton theorizes that friction with

the vessel wall, and hence significant effect due to vessel wall roughness, is not a factor in determining apparent viscosity.

Medium-size vessels. In blood vessels where diameter is of the same order of magnitude as the cell, blood does not behave as a homogeneous fluid; this change appears at vessel diameters less than 100μ. Axial accumulation occurs and a reduction in apparent mean viscosity would be anticipated.[154] The cells at the axis move slightly faster than the suspending plasma, with lowering of the mean hematocrit. Shear rate would be high at the relatively cell-free wall area; thus viscosity in this region is low. Between the cells the shear rate would be low, and thus the viscosity higher, a fact which may tend to stabilize axial flow.[148] It is known from the observations of Fåhraeus and Lindqvist[48] that with narrowing of tubes below 0.5 mm in vitro the viscosity of blood falls remarkably, a phenomenon thought to be due to both reduction of mean hematocrit in the vessel (plasma skimming) and also the fact that the cell thickness exceeds the thinnest laminae which could exist in blood.[67, 68] Bloch[8] has shown by high speed photomicrography that the "cell-free" marginal layer does in fact contain cells with interspersed plasma gaps in the region close to the wall (0.5μ) in distinction to Bayliss'[3,4] observations and those of Copley[31,32] that a 1- to 4μ cell-free immobile marginal layer exists. Any factor slowing flow would increase the apparent viscosity in such vessels.

In vessels approximately twice the erythrocyte diameter, Whitmore anticipates that the cells move face-to-face in a virtually plasma-free axial "core" in a continuous rouleaux with an effective viscosity approximately that of plasma, 1.3 centipoise (cp), a concept stated earlier by Wells and Merrill.[148]

Capillaries. Since the time of Rous'[114,115] observations, many investigators[8, 10, 11, 15, 48, 50, 63, 76, 81, 106, 125] have noted the remarkable deformability of the erythrocyte which permits its passage through capillaries smaller in diameter than the erythrocyte itself. When capillary diameter is less than cell diameter, the effective viscosity is calculated by Whitmore to approach infinity,[154] a condition obviously not true in real life, due to the thin plasma layer lubricating the plasma wall, and the marked flexibility of the erythrocyte itself. The capillary itself, due to its smaller diameter, is a physically very rigid, unyielding structure, as Burton[14] has pointed out; thus, extreme erythrocyte deformability is required at the prevailing shear stress to permit passage through such small channels. It is very important to observe that at very low capillary flow rates (significantly less than 1 mm/sec) viscosity may rise to extremely high values, and only when shear force is sufficient to exceed the yield stress will the erythrocyte move through the small vessel.

Capillary flow is known to be bolus flow, i.e., single file flow of cells with interspersed plasma.[100] No significant cell-cell interaction occurs, and the implications of fluid mechanisms, especially Poiseuille's law, have no significance. At flow rates of 1 mm/sec viscosity remains low (approximately 2 cp); in capillaries where diameter is greater than 5μ[50] a circular motion of the intracellular plasma is thought to improve equilibration of gases between capillary blood and tissues or alveoli,[100] as postulated by Roughton and Forster.[113]

Flow in channels of limiting diameter. In capillary vessels considered up to this point, single file flow of cells depends on the flexibility of the erythrocyte, and as Rand and Burton,[106] Guest, et al.,[63] Bloch,[8] and Branemark and Lindström[11] have shown, the erythrocyte may fold on itself or assume a projectile shape with

invagination of the trailing edge. If a vessel becomes sufficiently small, Canham and Burton[17] proposed that a limiting diameter will be reached through which the cell may no longer pass. This theoretical geometric parameter, or minimum cylindrical diameter, is described as the smallest right cylindrical channel through which the flexible cell might pass without increasing its membrane area.[17] To visualize the concept better one might consider a normal erythrocyte–8.3μ diameter, 90μ^3 volume, and 145μ^2 area–moving into a cylindrical channel, assuming the erythrocyte to be completely flexible, both in membrane and contents. In such a case, the minimum channel would be that having an area of 145μ^2, containing a volume of 90μ^3; if the diameter were less, the channel would be longer and the membrane would have to be "stretched" to cover the area. Although unidirectional stretch (i.e., increase in one linear dimension) occurs to values of up to 65%, forces tending to increase the area of the erythrocyte membrane cause hemolysis.[107]

Subsequent to Canham and Burton's[17] postulate of minimum cylindrical diameter (MCD), Gregerson, et al.,[62] employing thin polycarbonate filters, postulated an MCD of 2.8μ for normal human erythrocytes, for a channel approximately 12μ in length. Weed and LaCelle[140] have reported an MCD of 2.8μ observed in vitro by use of calibrated micropipettes. In channels smaller than 2.6μ, the channel length becomes of great significance. If these small channels are longer than 8 to 12μ, they become prohibitive; i.e., the erythrocyte, under hydrostatic force of the circulation, enters part way into the channel as a cylindrical projection conforming to the channel. However, the remainder of the cell remains outside as a spherical shape incapable of deformation. Additional force, if sufficient, may cause fragmentation; if not, the cell remains trapped.[78] But, if the channel is short (e.g., 4 to 5μ) a normal erythrocyte may "flow" through it, due to the extreme deformability of the normal cell. Such is thought to be the case when the normal erythrocyte passes through the relatively thin apertures 0.5 to 5μ in diameter[139, 143] observed in the basement membrane separating the splenic cords from sinuses, or in the bone marrow where hematopoietic cords are separated from the sinuses by the basement membrane apertures approximately 3μ mean diameter.[144] The narrow channels of the bone marrow, spleen, and other portions of the reticuloendothelial system are not simple right cylindrical passageways, and the effects of surface properties of endothelial cells on cell passage are not known. From the in vitro work of Seaman and Swank[123] and LaCelle,[78] it seems apparent that reduction of surface charge of erythrocytes by neuraminidase treatment does not alter the viscosity of these cells or their deformability as measured in a microcapillary.

Thus, it becomes apparent that in the smallest channels of the microcirculation, the flexibility of the erythrocyte and the dimensions of the channels are the most important determinants of flow characteristics, and rheologic properties of whole blood in larger channels may not always be predictive of cell behavior in limiting circumstances. It is predictable that small changes in erythrocyte properties in these restricted portions of the circulation may be crucial in determining whether cells pass through the channels. It is of note that the most restrictive portion of the circulation is the spleen; the MCD may increase approximately 10% postsplenectomy (theoretical MCD increased from 3.6 to 4.0μ postsplenectomy[17]). This suggestion is confirmed by the frequent clinical observation that a shortened erythrocyte life span increases after splenectomy.

It is important to recognize that the yield stress may become highly significant in small channels at low flow velocity, and the viscoelastic properties of the cell itself may thus cause it to have considerable apparent inertia to flow in channels of limiting size. Sudden application of force when the cell is in a small channel may greatly increase the intramembrane tension, leading to marked net rigidity of the cell.

Veins. In the postcapillary venules and sinuses flow velocities are slowest, and hence shear rates are below the value at which dispersion of cell-cell aggregates occurs. Viscosity is relatively high, particularly if intermittent flow is present. The aggregates contribute significantly to the yield stress of the blood, and, dependent on the erythrocyte deformability, considerable inertia may exist. Functional occlusion may occur, as noted below (p. 22). In venules where shear rates are 0 to 50 sec^{-1}, aggregation and erythrocyte deformability will determine viscosity, and in larger vessels where shear rates approach 150 sec^{-1}, aggregation and shear dependence will decrease.[153] However, since shear rates are in the range where aggregation is significant, any process slowing flow will adversely affect viscosity.

Contribution of Blood Components to Blood Rheology

Viscosity of pure water is difficult to determine because of its surface tension[90] and surface charge characteristics in a viscometer; however, water containing the electrolytes typical of blood has a viscosity similar to the absolute viscosity of pure water and is Newtonian. Larger molecules alter viscosity, and it is evident that fibrinogen[89] is the chief molecule contributing to the plasma viscosity and the cell-cell interaction typical of rouleaux formation, which phenomenon contributes to whole blood viscosity at shear rates less than 50 sec^{-1}. Chien and co-workers indicate[30] that α and β globulins may enhance fibrinogen-induced cell-cell aggregation. Merrill[90] reports a decrease in yield stress and anomalous viscosity of blood from afibrinogenemic patients and increase in both parameters in hyperfibrinogenemic blood (600 mg/100 ml fibrinogen).

The erythrocytes themselves contribute as the major determinant of the net rheologic properties of blood,[30] as the result of cell-protein interaction which is responsible for aggregation and rouleaux formation, and also as the result of cell-cell interaction in the absence of protein. When erythrocytes are washed free of plasma protein in pH 7.4 buffer and viscometric determinations made, the effects of cell-cell interaction and deformability of the cells are evident as the only factors determining viscosity. As Burton[14] has emphasized, the viscosity of close-packed erythrocytes depends on the deformability of the adjacent erythrocytes, i.e., their ability to slip by each other. The fact that more than 60% hematocrit is achievable at rest or during flow is evidence that erythrocytes are deformable, for rigid biconcave discs with the dimensions of erythrocytes could only be packed theoretically to a hematocrit of 58%.[14] Experimental findings indicate marked decrease in packing of erythrocytes hardened with acetaldehyde.[25] Thus, erythrocyte deformability is a highly significant determinant of whole blood viscosity.

It is rarely appreciated that viscosity varies very little with hematocrit over the range of 0 to 40%, as is indicated in Figure 2. The yield stress is small over this range.[90] The relative increase of viscosity at 50% hematocrit as compared to that at 25% is insignificant in the circulation. At regions where cell-cell interaction is important shear rates are relatively high and cells do not aggregate, while at regions of

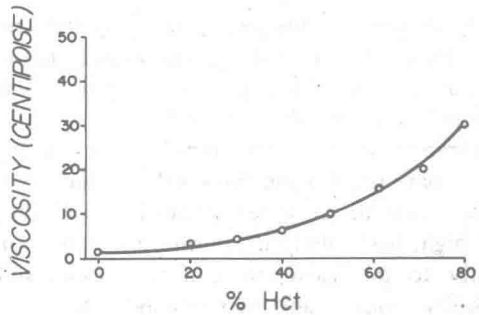

FIG. 2.–Changes in whole blood viscosity with hematocrit.

low shear the cell-cell interaction is negligible. Above 50%, increasing the hematocrit causes more significant changes in viscosity, particularly at lower shear rates.[89, 90]

In hypotonic plasma, the yield stress of blood has been reported to increase and viscosity to decrease, whereas hypertonic media decrease the yield stress and increase viscosity.[86] These findings appear to result from changes in the cells themselves (swollen biconcave discs in hypotonic medium and crenated discs in hypertonic medium). Rand and Burton[106] and Schrier and co-workers[121] reported that swollen cells in hypotonic media are more viscous than normal. Crenated cells in hypertonic media also have greater viscosity.[64, 118]

Interpretation of viscometric data is complicated by the existence of several types of viscometers, variable conditions under which measurements are made, and uncontrolled artefacts in the measuring systems. Capillary viscometers are inappropriate for extrapolation of in vitro phenomena to in vivo circumstances, since such viscometers have high fixed shear rates, often much higher than physiologic values. The cone-plate viscometer as described by Wells,[147] and the GDM Couette cylinder-in-cylinder viscometer[57] used by Meiselman, et al.,[86-89] and Chien, et al.[25-30] permit viscosity measurements at varied shear rates. Many other viscometers, such as those devised by Dintenfass[34-43] attempt to avoid the problems of artefact due to cell sedimentation with a resulting two-phase system, syneresis of plasma next to the boundary surfaces and increase of density of the cellular core.[90]

RHEOLOGIC CHARACTERISTICS OF THE NORMAL ERYTHROCYTE

Cell shape, the mechanical properties of the membrane, and the physical state of the cell contents determine the rheologic characteristics of the erythrocyte. Alteration of any of these determinants is sufficient to decrease the cellular deformability, and, as postulated above, small changes of cellular deformability may have marked effects on the capacity of such altered cells to pass through restrictive channels of the microcirculation.

Significance of the Biconcave Disc Shape

For over 50 years scientists from different disciplines have discussed the typical biconcave discoid shape of the human erythrocyte, and in recent years Ponder[99] and Lehmann and Huntsman[81] have reviewed critically the various proposals of mechanisms to account for this unique shape. Fung[51] has examined considerations of shape

from the standpoint of theoretical mechanics, and Canham,[18] combining theoretical and experimental results, concludes that the concept of minimum energy of bending, i.e., the principle of least total curvature of the membrane, is adequate to explain the erythrocyte shape. From the observation of Branemark and Lindström[11] indicating that erythrocytes rapidly resume their biconcave shape after leaving narrow capillaries, and from the observation of Rand and Burton,[106] and Weed, et al.[141] that erythrocytes return to the normal biconcave shape after extrusion from narrow glass capillaries, the implication that the biconcave disc shape requires the minimum energy intuitively appears valid. The minimum energy of bending, of course, does not refer to energy derived from intracellular metabolic processes, but instead to energy denoted within the framework of theoretical mechanics.

Ponder[99] observed the biconcave disc shape of the ghost, and it is known that an erythrocyte ghost containing no unhydrolyzed adenosine triphosphate (ATP) maintains a biconcave disc shape in the presence of suitable ion concentration.[69] Canham's[18] concept of minimum energy of bending is at odds with Fung and Tong's[52] conclusion that the minimum energy is negligible, and that an equatorial specialized area of tensional stiffness accounts for cell shape. Both Rand and Burton[106] and Weed, et al.,[141] employing micropipette techniques, were unable to find areas of increased resistance to deformation; however, the micropipette techniques may not possess sufficient sensitivity to detect the extremely small variations in local membrane composition that determine cell shape. Murphy[91] has proposed equatorial localization of cholesterol as an explanation for the biconcave disc shape, but this work has been criticized by Fung and Tong.[52] Localization of excessive cholesterol in a bilipid membrane might actually decrease stability.

It appears likely that the shape is a net result of the equilibrium between surface tension, ratio of cell radius to membrane thickness, pressure differential across the membrane, and extensional stiffness of the membrane, assuming the erythrocyte membrane is in fact elastic, an assumption supported by the recent work of Blais and Geil in red cell ghosts,[7] and by the data of Rand and Burton[106] and Katchatsky.[74]

Regardless of the specific determinants of the biconcave shape, this shape, possessing excess area for the enclosed volume, is uniquely advantageous to the cell in permitting remarkable deformability. As long as the cell contents are liquid, a fact supported by the observations of Fung,[51] Rand and Burton,[106] Dintenfass,[35, 41, 42] and Wells and Schmid-Schönbein,[150] this red cell geometry implies that the membrane is capable of deforming into an infinite number of applicable (isometric) surface configurations without stretching the membrane and without volume change. Thus, it is predictable that the membrane could, as has been observed, withstand great bending stresses without inducing membrane stresses (leading to increased membrane tension, compression, or shear and resulting in membrane stiffness or rupture) and without changing the pressure differential between cell interior and the external suspending medium.

Membrane Viscoelastic and Plastic Properties

In addition to its viscoelastic properties,[74, 106] the membrane in some cases contains a plastic element,[145] i.e., a constituent which, if distorted sufficiently, will not reversibly regain its initial dimensions and physical characteristics. Poikilocyte formation may be a result of distortion of a membrane plastic element.

Experimental data characterize the membrane as a viscoelastic material having a plastic element. Other observations indicate that the rheologic character is complicated by thixotropy[34, 145] (the tendency for decreased resistance to deformation as strain rate increases) and in some cases by dilatancy or rheopexy[145] (resistance increases with increasing rate of force application). These latter properties may vary nonlinearly with strain rate and duration of stress.[34]

Fluidity of Normal Cell Contents and Shear Dependent Fluid Transition

It is known that the erythrocyte membranes can transmit shear stress to the cell contents as predicted by Fung[51] and shown by Dintenfass.[35, 41, 42] Schmid-Schönbein and colleagues have observed cell behavior by direct visualization of cells in the shear field; they have documented shear deformation of the cells and fluidity of the normal cell contents.[116, 119, 150] They postulate that the cell contents under shear transmitted by the membrane become liquid and that the erythrocyte has relatively low viscosity (*viz.* 6 cp) and is Newtonian in behavior. Schmidt-Nielsen and Taylor[120] have also noted that viscosity of membrane-free dog and goat hemoglobin (concentration less than 30 g/100 ml) is less than that of intact normal cells; however, in bovine hemoglobin concentrations greater than 20 g/100 ml, the solution has gel characteristics, a fact which would induce altered rheologic characteristics. (It is virtually impossible to achieve aqueous solutions of human hemoglobin in concentration of 32 g/100 ml).

Interrelationships of Shape, Membrane Deformability, and State of Cell Contents

Shape, intrinsic membrane deformability, and the fluidity of cell contents, the major determinants of erythrocyte deformability, are interdependent. The extreme flexibility of the cell as a function of its biconcave shape is dependent on the intrinsic deformability of the membrane and fluidity of the intracellular hemoglobin solution. Fluidity of contents in a shear field requires a deformable membrane which transmits force to the cell interior. Shape, of course, may be a function of cell content (e.g., sickle hemoglobin at low oxygen tension) and also of the membrane (e.g., spherical shape at low intracellular ATP concentration[95, 141]).

Shape is an important parameter, for a sphere is recognized to be a geometric form of considerable rigidity. For example, a spherical erythrocyte ghost derived from a normal cell is more rigid than the biconcave disc-shaped ghost; in each, the contents of the ghosts are isotonic salt solutions with η equal to 1 cp. It is not possible to differentiate entirely the contribution of intrinsic membrane deformability to net cell deformability from the contribution of cell shape to overall cellular deformability. Alteration of shape to a spherical configuration is insufficient as the sole explanation for altered erythrocyte deformability during the disc-to-sphere transformation, for, as Merrill[88] observes, alteration of either a rigid or nonrigid and anisodiametric particle to a spherical configuration of equal volume should result in reduced viscosity.

MECHANISMS THAT ALTER ERYTHROCYTE DEFORMABILITY

Adenosine triphosphate (ATP) is widely recognized as the source of high energy phosphate requisite to the maintenance of normal intracellular cation composition in the erythrocyte by means of an ATPase-related active cation transport mechanism. ATP is also essential, as Nakao and collaborators[94, 95] observed, for the preservation