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The Internal Viscosity of the Red Cell and the Structure of the Red Cell Membrane. Considerations of the Liquid Crystalline Structure of the Red Cell Interior and Membrane from Rheological Data

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Abstract—In a series of experimental studies, carried out by means of rotational and microcapillary viscometers, it was possible to show that the viscosity of packed human cells, at haematocrits up to 98 per cent, is in the range from 10 to 1,000,000 centipoises, depending on the shear rate and on the type of the red cell (i.e., the normal red cell, the sickle cell, crenated cells, etc.). The viscosity of blood, or of suspensions of the red cells, is also very low and requires an acceptance of the red cell as a (complex) fluid drop. A study of suspensions of red cells in the continuous phases of different viscosities (i.e., blood plasma, saline, dextrane solutions) and osmolalities confirms that the internal viscosity of the red cell must be very low indeed.

An equation, which is based on the Roscoe, Brinkman and Taylor equations, and which reduces at low concentrations to Taylor's equation, is applied in order to define the internal viscosity of the red cell. This is found to be between 1 and 6 centipoises for the normal cells and up to 20 or more centipoises for the abnormal red cells. These values include the contribution of the redcell membrane. Utilizing equations of Oldroyd and Boussinesq, the surface viscosity of the red cell is estimated to be between 0.000001 and 0.000004 surface poises. An attempt is made to reconcile these values with the data of Katchalsky and Burton and Rand. Such reconciliation is feasible if the viscosity of the membrane is very high at high stresses and/or during haemolysis; and if the elastic modulus is also proportional, in some manner, to the stresses applied. It is suggested that the membrane is of thixotropic-dilatant type with a non-Hookean elastic

component; a non-linear viscoelastic body which is time-dependent.

A molecular structure of such membrane could be composed of two main phases: one which would be liquid crystalline, and the second which would be formed of a loose and dynamic network of proteins. A multiphase, complex, dynamic, heterogeneous and liquid crystalline membrane would be compatible with the current ideas on the structure of biological membranes, on the active transport and on the catalytic reactions within the cell membrane.

Introduction

The rheological and molecular properties of the red cell, and its membrane in particular, are of great interest and, indeed, speculations on these properties occupied scientists as far back as beginning of 17th century. A concept of the red cell as a balloon-like body, in which fluid solution of haemoglobin and salts is enclosed by an external envelope, was suggested by Bidloo in 1685.[1] This concept was defended later[1] by Schäfer against Rollet who, in 1862, described the red cell as composed of colorless matrix or stroma in the meshes of which the haemoglobin is embedded. Subsequent discussions between advocates of the "balloon" theory and those of the " gelatine lozenger " theory were not able to decide this matter one way or the other.[1]

Viscometric studies of blood by Hatscheck,[2] in early 1920, and much later by Bingham and Roepke,[3,4] suggested that the interior of the red cell must be mobile and that blood might be considered an emulsion. This work was neglected and forgotten later on, and in the middle fifties—when a new generation of physicists entered the field of blood rheology—it was assumed that blood, alike all the other suspensions, shows an infinitely high viscosity at high haematocrits, and that the internal viscosity of the red cell is of no consequence. However, anyone connected with the practical haematology knows that the packed red cells, of haematocrits up to 98 per cent, remain quite fluid.

Particular attention has been given to the chemistry of the red cell, and the most modern techniques, including polarization microscopy, electron microscopy, X-ray diffraction, and nuclear

magnetic resonance spectroscopy, and other techniques[5] have been used to elucidate the structure of the red cell membrane. The famous Danielli model of the membrane, composed of a bilayer of lipids and proteins, has been elaborated in Robertson's unit membrane hypothesis[6,7] and further elaborated as macromolecular repeating units.[8] Unfortunately, these studies and concepts were based on techniques using ghosts or dead (frozen, dried, etc.) red cells and it could be suspected, and even anticipated, that the properties found in each case would depend on the method of preparation and the investigational technique employed.

Using non-destructive, viscometric techniques, it will be shown that the internal viscosity of the red cell is very low, and some tentative equations will be given to illustrate this point. The very fact of the low internal viscosity of the red cell requires a two-phase system for the cell interior. From the consideration of these equations it will appear that the surface viscosity of the red cell membrane is very low. An effort will be made to reconcile the data obtained with the data of other investigators, and it will be suggested that the cell interior and, especially, the cell membrane are multiphase liquid crystalline systems of variable orientation and configuration of components, and of variable viscosity and elasticity.

Experimental

A. Instrumentation

Viscometers employed in this study included the cone-in-cone and the ring-in-ring rotational viscometers,[9,10,11] the newly developed rhombo-spheroid viscometer, and the parallel-plate slit capillary viscometers.[12,13] The rotational viscometers contained a suspended member (cone, ring or rhombospheroid) made of Teflon, and an outer, rotating, member made of brass. Torsion strips made of " Mallory 73 " beryllium copper alloy were employed. Deflection of the torsion strip, and of the inner member

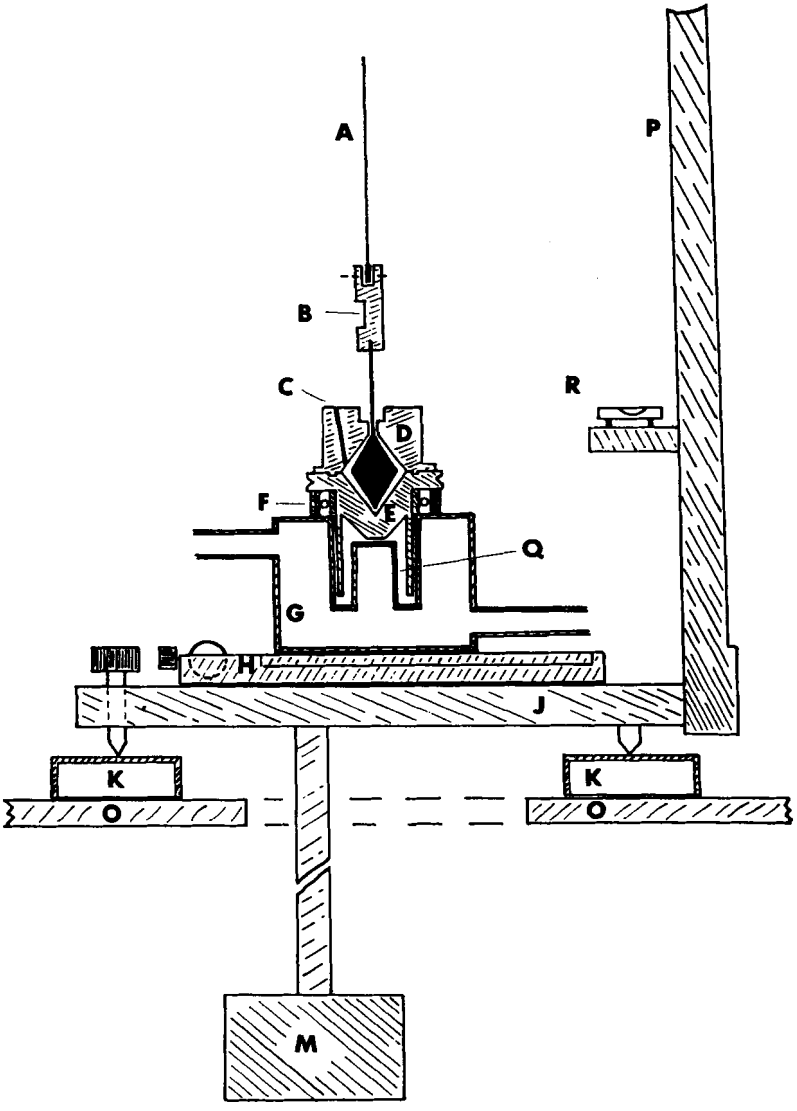
was observed by means of a galvanometer mirror and a " Cambridge " spotlight, a lightspot being reflected onto a semicircular plastic scale. In the modified instrument, the thermostated cup, which encloses the coaxial unit, is situated on the microscopic stage in order to facilitate centering of the inner and outer members (Fig. 1). Calibration of the viscometers was carried out using Newtonian oils of known viscosity. These were obtained from the National Standards Laboratories, C.S.I.R.O., Sydney. Shear rates were calculated as described earlier.[14]

The slit microcapillary viscometers were formed of two highly polished glass plates separated at the edges by thin plastic films (Fig. 2). Series of plates were used, in which the gap varied from 5μ to about 100μ. The size of the plates was selected in such a manner that the efflux times were not unreasonably long; the slit capillaries were up to 15 cm long and up to 20 cm wide. Changes in the pressure gradient were obtained by moving the pipettes up or down. The pipettes were connected, through plastic tubing of 2 mm diameter to the horizontal inlet and outlet channels of the parallel-plate slit capillary. The slit capillaries were calibrated using distilled water. A thermostated tank was available.

B. Materials

The packed blood cells have been prepared from the human blood, freshly collected and anticoagulated with EDTA or acid

Figure 1. The rotational coaxial rhombo-spheroid viscometer, a further modification of the original cone-in-cone viscometer. *A:* a copper beryllium torsion strip; *B:* a galvanometer mirror reflecting a lightspot onto a semicircular scale; *C:* a channel for insertion of a hypodermic needle by means of which the space between the rhombospheroids is filled with blood; *D:* the upper part of the rotating member; *E:* the lower part of the rotating member, equipped with brass wings for better heat transfer; *D* and *E* enclose the suspended member indicated in solid black. *F:* roller bearing; *G:* thermostated jacket; *Q:* oil bath; *H:* a microscopic stage used for centering of the coaxial rhombospheroids; *J:* the steel base of the viscometer, equipped with a heavy (35 lbs) keel, *M*, for improved stability; *K:* shock absorbers; *O:* the top of the trolley; *P:* a steel mast carrying the torsion strip, and adjusted in vertical position while checking the spirit levels *R*.



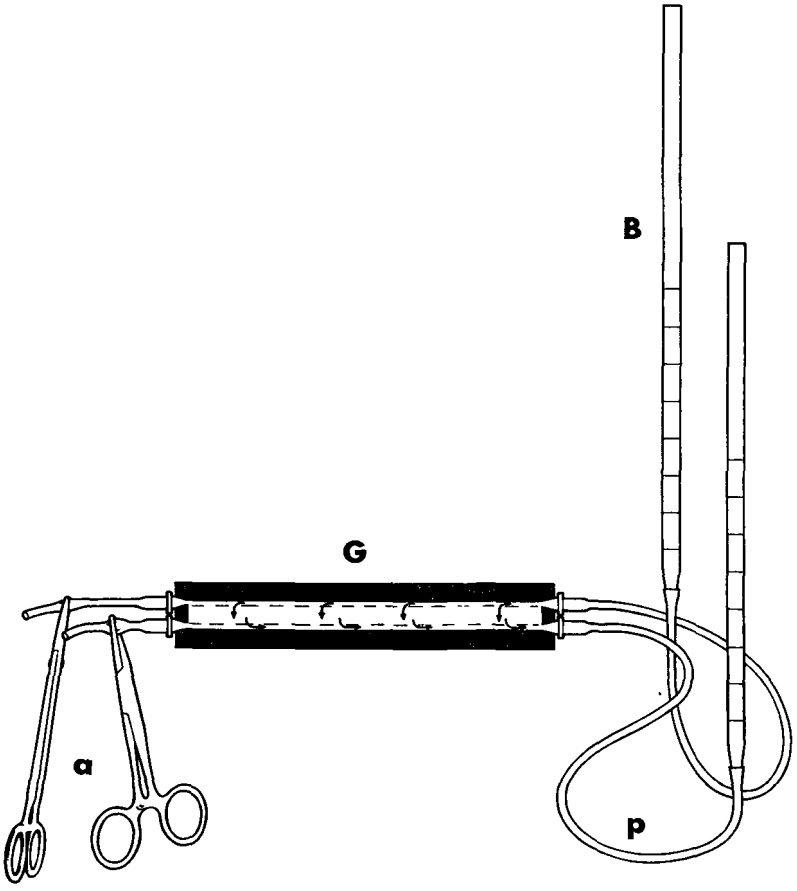


Figure 2. The parallel-plate slit capillary viscometer. The viscometer is constructed from two glass plates, *G*, containing two horizontal inlet and outlet channels. These channels are connected by means of plastic tubes, *p*, with calibrated 1 ml pipettes, *B*. The channels are equipped also with plastic tubes on the other end (closed with arterial clips *A*) but these are for cleaning purpose only. Blood flows like a sheet from the upper to the lower horizontal channel. Pressure drop is adjusted by moving one of the pipettes up or down.

citrate dextrose. Blood was centrifugated, the supernatant plasma removed, and cells washed a few times and repeatedly centrifugated either in the isotonic, hypertonic or hypotonic saline or in solutions of dextrane of various viscosities and tonicities. Solutions of dextrane were prepared by dissolving 3 to 25 per cent of 200,000 molecular weight dextrane in water; tonicity being adjusted with sodium chloride. In all tests the viscosity and the tonicity of the supernatant was checked.

Concentration of the red cells in all samples of blood or packed cells was determined by means of " Hawksley " Microhaematocrit Centrifuge. Haemoglobin determinations were made using cyanmethaeglobin method in the " Unicam SP1300 " colorimeter. Haematocrits and haemoglobin results were compatible; there was no effect of the plasma or dextrane viscosity on the packing of the red cell in excess of 2 per cent.

Blood from the patients suffering from Waldenström macroglobulinaemia was obtained either in this laboratory, and then a solid anticoagulant (EDTA) was used, or obtained from the Department of Haematology, and then a liquid (ACD) anticoagulant was used.

On two occasions blood was obtained from patients suffering from the sickle cell disease. Some samples of normal red cells were treated with formalin which crosslinks protein in the red cell. A 7 per cent solution of formalin in saline was used, each treatment lasting for half an hour; after each treatment cells were washed with isotonic saline. This procedure resulted in red cells remaining monodisperse.

In the case when blood, or suspensions of the red cells, were intended for use in the slit microcapillary viscometer, great care had to be taken to remove white cells and platelets (the buffy coat) and blood was examined under microscope before the viscometric test.

Results

Results of this study are contained in a series of tables and graphs (Tables 1–4, and Figs. 3–9). Basically, they can be

divided into the following series: (a) a series on which internal viscosity of the red cells depends on chemical structure of the cell interior: here belong sickle cells, containing haemoglobin SS or SA; normal cells, which contain haemoglobin AA; or cells treated with formalin; (b) a series in which the size of the cells and the internal concentration of haemoglobin is varied by use of saline or dextrane solutions of different osmolality, but mainly high osmolality which results in formation of crenated cells; (c) a series in which the viscosity of the continuous medium (i.e., plasma, dextrane solutions, etc.) is varied in order to yield the effect of this parameter on the viscosity of blood, and thus to permit an evaluation of the effect due to the internal viscosity of the red cell; (d) a series intending to define minimum viscosity of blood or suspensions of the red cells.

The experimentally obtained data are utilized in calculations of the internal viscosity of the red cell and of the viscosity of the red cell membrane.

However, in order to obtain a logical picture, it is necessary to utilize published data of various investigators; some of these are contained in the text of this paper, other are presented in the form of tables (Tables 5 and 6).

Two crucial results are obtained: (a) that the viscosity of blood and suspensions of red cells can be, even at red cell concentrations of 98 per cent, as low as 2 to 20 centipoises (while suspensions of rigid particles will show consistency of a brick already at about 65 per cent concentration); and (b) that the relative viscosity of blood is related inversely to the viscosity of plasma (while viscosity of suspensions of rigid particles is always directly proportional to the viscosity of continuous phase).

Discussion

The peculiar property of the concentrated suspensions of the normal human red cell (the packed red cells) to exhibit very low viscosity, in striking contradistinction to the suspensions of rigid

particles, forms the basic fact from which all the subsequent considerations must originate. This basic fact was recognized in 1962 by Dintenfass[15] who utilized the then available literature data. In this study this basic fact is experimentally confirmed, and, what is more important, a second basic finding is added: that the relative viscosity of suspensions of the red cells is related in an inverse manner to the viscosity of plasma, and thus behaves as a suspension of fluid drops obeying, in principle, the theory of Taylor.[16] The latter, and its elaboration by Oldroyd,[17,18] will be employed to deduce the physico-chemical properties of the red cell membrane.

Consequently, the discussion is divided into three main parts; in the first part the experimental data are utilized to obtain numerical values of the apparent internal viscosity of the red cell and to deduce the microstructure of the cell interior; in the second part the rheological characteristics of the red cell membrane are deduced using Oldroyd[17] and Boussinesq[19] equations; in the third part the concept of the liquid crystalline membrane is discussed and the literature data and information used for elaboration of such a concept.

1. The Apparent Internal Viscosity of the Red Cell and the Structure of the Interior of the Red Cell

Suspensions of the red cells, the packed cells, or blood, show very low viscosities in relation to the concentration of the disperse phase (the red cells). If tested over a range of shear rates, it is a characteristic feature of these systems that the viscosity de-decreases as the shear rate increases. While in more dilute suspensions such phenomenon is attributed to the aggregation of the red cells, in the systems of tightly packed red cells (haematocrit 98 per cent) such viscosity decrease must be due to the cell itself (its interior and its membrane) and not due to aggregation of these cells.

While suspensions of rigid particles reach infinity[20,21] at concentrations above 65 per cent, the normal red cells can easily form suspensions containing volume concentration of 98 per cent of the

red cells. This ease of packing is a characteristic feature of the deformable and fluid particles. Indeed, the low viscosity exhibited by packed cells could not be explained unless the red cell is considered as a fluid particle.

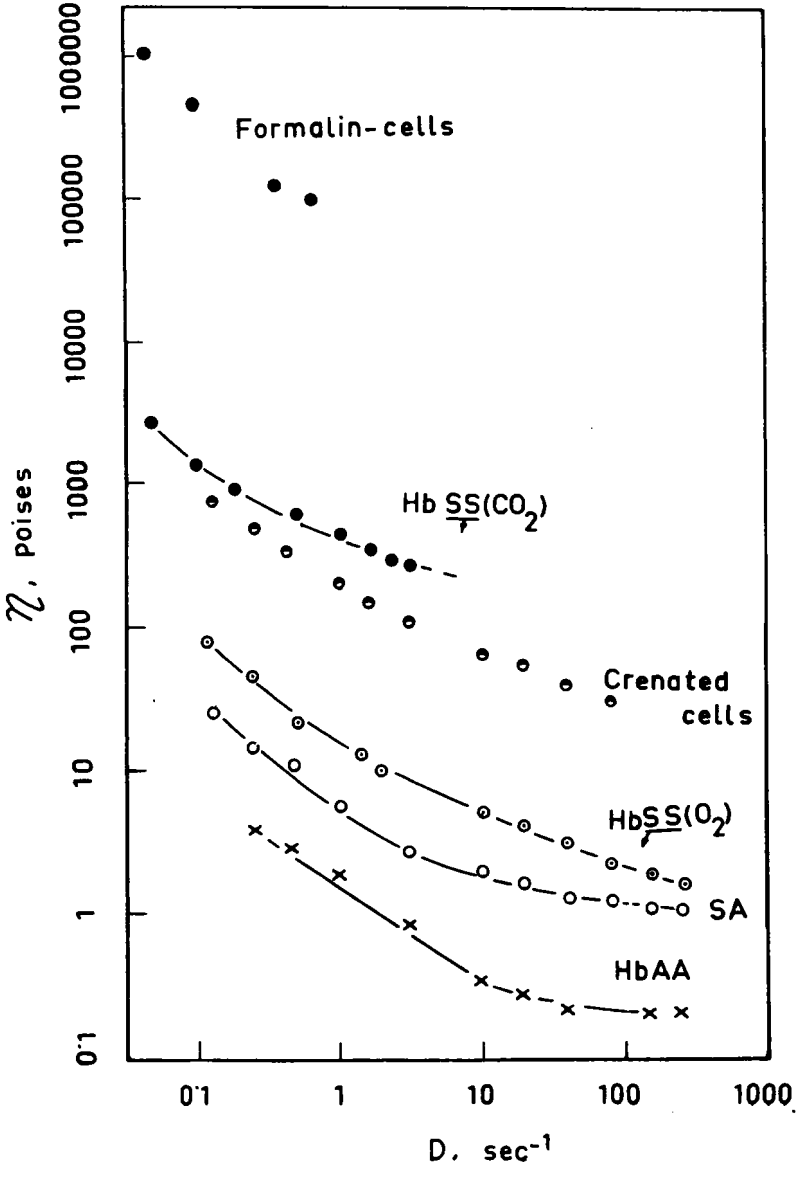
As can be seen from the experimental data (Fig. 3, Table 1), this fluidity can be affected by crenation of the red cell, by the type of haemoglobin present, or by polymerization of proteins

TABLE 1 Relative Viscosities of Blood in Plasmas of Different Viscosities

Haematocrit %	Plasma viscosity, cps.	Relative tonicity	Relative viscosity of blood, η_r, at 44 sec^{-1}
80	0.7	0.57	220
32	0.7	0.57	20
85	10.7	0.57	20
30	10.7	0.57	1.2
65	0.7	2,76	37
38	0.7	2.76	9.7
70	6.5	2.73	12.7
35	6.5	2.73	2.24
58	0.9	1	6.2
60	1.6	1	5.5
58	3.3	1	3.3

within the red cell using formalin. The latter may show viscosities (in suspensions) about 100,000-fold higher than the packed normal red cells; crenated or sickle cells may show viscosity (in the form of packed cells) about or up to 500-fold higher than the packed normal red cells.

Figure 3. Viscosity of the packed human red cells. Viscosity, η, in poises, is plotted against the rate of shear, D, sec^{-1}. All systems show haematocrit of 98 per cent. Formalin cells: red cells treated with formalin; HbSS(CO_2): suspensions of red cells containing haemoglobin SS under reduced condition; Crenated cells: suspensions of red cells from normal adult blood in hypertonic saline; HbSS(O_2): suspensions of red cells containing haemoglobin SS when oxygenated; HbSA: suspensions of red cells containing haemoglobin S and A; HbAA: suspensions of normal adult red cells.



The new and additional characteristic feature of the suspensions of the red cells is that the relative viscosity of these suspensions decreases as the viscosity of the continuous medium increases (Figs. 4–8, Table 2). Actually, a single series showing exactly this behavior has been described in 1965, but could have gone unnoticed by physicists, as it was contained in a paper[22] published in the journal of *Experimental and Molecular Pathology*.

TABLE 2 Estimation of the Internal Viscosity of the Red Cell from Viscosity of Blood in Plasma of Different Viscosities. Isotonic Systems at 37 °C

Haema-tocrit %	Plasma η_0 cps.	Blood η_r	Shear rate, sec^{-1}	T	p	Internal viscosity of the red cell, η_i, cps.
98	0.7	146	60	0.885	4.15	2.9
98	0.7	134	80	0.875	3.85	2.7
38	9.6	1.75	72	0.527	0.27	2.6
38	13.5	1.70	72	0.50	0.20	2.7
98	0.7	130	100	0.874	3.75	2.7
98	1.1	98	190	0.857	3.15	3.50

This behavior of blood, or suspensions of the red cell, is most significant as it is the basic rule of rheology of suspensions of rigid particles that suspensions viscosities are directly proportional to the viscosity of the continuous phase, other parameters being constant. Every viscosity equation, starting from the well known Einstein equation and all subsequent ones, accepts it as a fundamental rule. This rule has been proved in experimental studies, i.e., over 7000-fold variation in the viscosity of the continuous phase.[23]

The reason why blood, or suspensions of the red cells, disobey this rule is due to the simple fact that the red cell behaves as a fluid drop, and blood behaves as an emulsion which viscosity depends on the ratio of the internal viscosity of the drop to the viscosity of the continuous phase.

Equation for dilute emulsions was given by Taylor[16]:

$$\eta_r = 1 + 2.5C\frac{\eta_i + \frac{2}{5}\eta_0}{\eta_i + \eta_0} \tag{1}$$

or

$$\eta_r = 1 + 2.5CT \tag{2}$$

in which T, Taylor's constant, can be given as $(p + 0.4)/(p + 1)$, where $p = \eta_i/\eta_0$; here, η_0 is the viscosity of the continuous phase, η_i is the viscosity of the drop interior, C is the volume fraction of the disperse phase, and η_r is viscosity of emulsion divided by the viscosity of the continuous phase.

Taylor's equation takes into account the internal circulation in the suspended fluid particles. This circulation is due to the transmission of the tangential and the normal stresses across the interface between the continuous and the disperse phases during shear flow. Fluid circulation inside the drops reduces the distortion of the flow pattern outside the drops and reduces hence the volume fraction of the disperse phase by a factor T, the Taylor's factor; that is, the term C in the equation is replaced by a term CT.

Theoretical and experimental studies on the internal circulation in drops have been carried out by Mason and his collaborators[24,25,26] in Canada. Mason was able to relate the mean periods of internal circulation to the shear rate and to the Taylor's coefficient T. This might be of importance in studies dealing with oxygenation of red cells, and because of it Mason's equations are here reproduced:

$$\frac{M_c D}{4\pi} = \frac{2(p+1)}{((2p+5)(2p-1))^{0.5}}$$

$$\frac{M_{\text{int}}}{M_{\text{per}}} = 2\left[\frac{p(p+2)}{(2p+5)(2p-1)}\right]^{0.5}$$

in which M_c is the mean period of circulation, M_{int} is the period of more inner circulation, and M_{per} is the period of the peripheral circulation. (In the original work the symbol for circulation was T, but here it is replaced by M in order to prevent confusion with Taylor's constant).

It becomes obvious, and it was stated already many times by earlier investigators, that when the internal viscosity of the drops becomes greater than 50-fold viscosity of the continuous phase, such drops will behave as rigid particles. Consequently, if the packed cells behave as fluid drops, then the internal viscosity of the red cell must be greatly less than the product of viscosity of the saline and factor of 50; that is, less than 35 centipoises.

A direct application of the Taylor's equation is not feasible, as we are dealing with concentrated, or very concentrated, suspensions of the red cells. Taylor's equation is valid only for very dilute systems of concentrations up to few per cent of the disperse phase.

Consequently, it was necessary to develop a new equation which would be able to account for viscosities of very concentrated emulsions, and specifically of monodisperse emulsions.

Such an equation can be deduced along the lines used by Roscoe[20] or Brinkman[27] for the concentrated suspensions of rigid spheres. It is assumed that at each level of concentration of the disperse phase, the emulsion can be replaced by a continuous fluid of equal viscosity; after addition of a small aliquot of the disperse phase, this new emulsion will obey Taylor's equation; at each subsequent addition of drops, the earlier emulsion is treated as a continuous fluid of equal viscosity.

Roscoe–Brinkman equation for rigid spheres:

$$\eta_r = \frac{1}{(1 - kC)^{2.5}} \tag{3}$$

The new equation for fluid drops:

$$\eta_r = \frac{1}{(1 - kCT)^{2.5}} \tag{4}$$

In the Roscoe's equation the coefficient k was introduced to take account of the immobilization of the continuous phase (i.e., stagnancy). Roscoe suggested that the value of coefficient k is 1.35 for suspensions of spheres of equal size, but only 1.0 for suspensions of spheres of infinitely variable size. In the case of blood, the value of coefficient k might be related to the degree of plasma trapping.

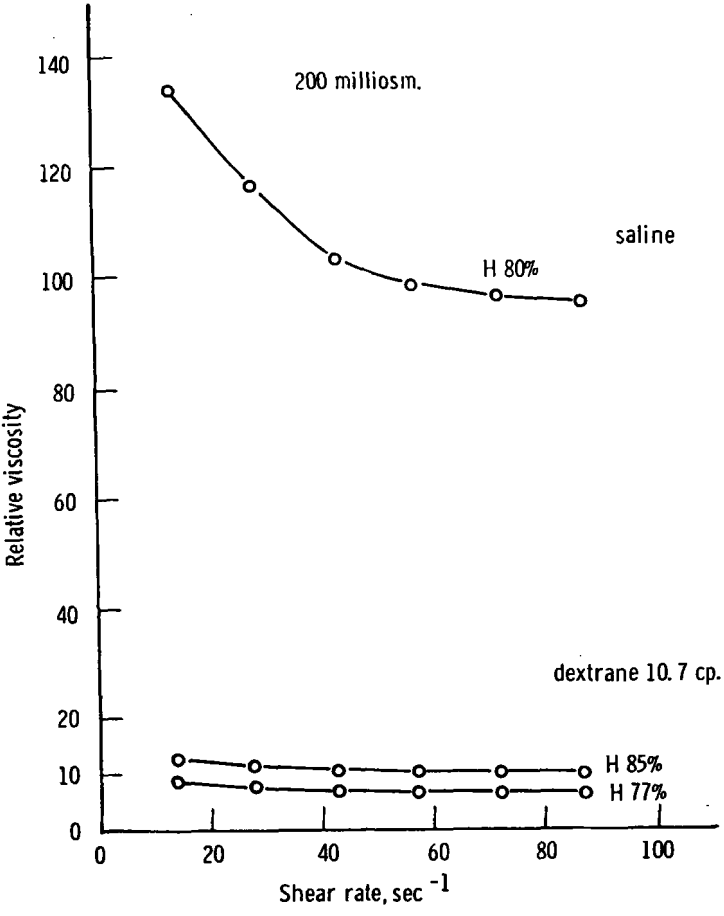


Figure 4. Viscosity of hypotonic suspensions of red cells in saline and in dextrane solutions. Relative viscosity (viscosity of suspension divided by the viscosity of the continuous phase) is plotted against the shear rate. Viscosity of dextrane solution is 10.7 centipoises. *H:* haematocrit.

Series of investigators attempted to define plasma trapping factor in blood.[28] Plasma trapping factor is usually given as 3 per cent (that is, $k = 1.03$) for the normal blood studied under the usual laboratory conditions, that is at low blood pH. For pathological blood, the plasma trapping factor can be larger, and i.e. for the sickle cells it might be as large as 10 to 20 per cent (Table 3).

TABLE 3 Calculation of the Values of Taylor's Constants T and the Apparent Internal Viscosities of the Red Cell from the Experimental Viscosity Data

	Type of the haemoglobin in the packed cells (Hct 98%)		
	AA	SS_{O_2}	SS_{CO_2}
η_r	20	158	1600–16000
T	0.715	0.886	0.967–0.979
p	1.1	4.2	17–27
k	1.01	1.04	1.1–1.2
C	0.97	0.94	0.89–0.815

η_r is the relative viscosity as determined by rotational viscometry;
k is the packing coefficient according to the literature data (see text).

It is realized that plasma trapping depends on the speed of centrifugation[29] as well as on the size of the cell[30]; and obviously, on the deformability of the cell. This could be further complicated by the interrelationship of the viscosity of the cell interior and the viscosity of plasma. Additionally, it is recognized that as the shear rates are reduced, both the rigidity of the cell and the packing factor (or plasma trapping factor) might increase.

It is assumed, however, that haematocrit, as measured in the high-speed microhaematocrit machines, could supply a good indication of the value kC, especially in relation to systems under higher shear rates. Were this correct, then we could write Eq. (4) as follows:

$$\eta_r = \frac{1}{(1 - HT)^{2.5}} \tag{5}$$

in which H is haematocrit expressed as a volume fraction.

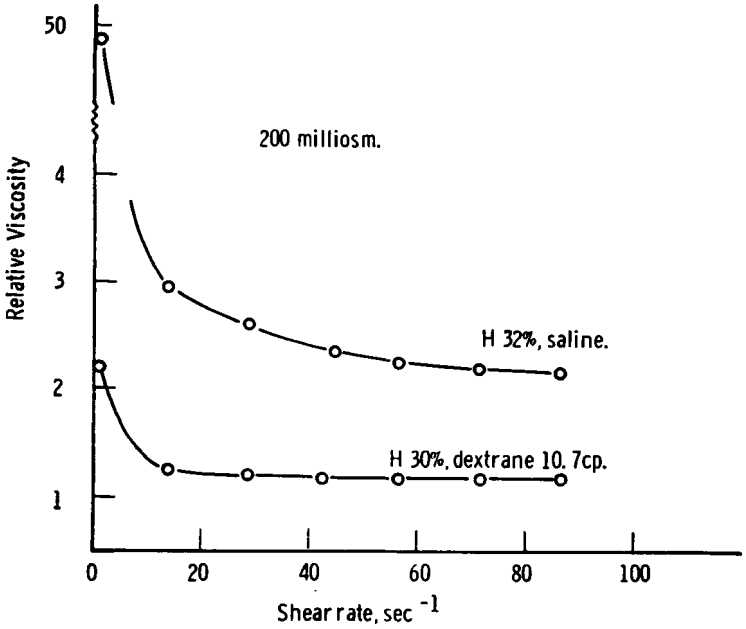


Figure 5. Viscosity of hypotonic suspensions of red cells in saline and in dextrane solutions. Relative viscosity is plotted against the shear rate. Viscosity of the dextrane solution is 10.7 centipoises. *H:* haemotocrit.

As our aim is to define the minimum values for the apparent internal viscosity of the red cell, we can restrict ourselves to studies at higher shear rates. An additional advantage of higher shear rates is that the red cells are monodisperse; aggregation of the red cells would lead to an increase in plasma trapping and an increase of the packing factor k.

Equation (5) has been utilized to obtain the apparent internal viscosities of the red cells (Tables 1–4). It can be seen that the apparent internal viscosity can be as low as one centipoise. If the order of the internal viscosity of the red cell is just about the viscosity of the blood plasma, then any changes in the viscosity of plasma should have a marked effect on the p and T. This is exactly what happens (Figs. 4–10; Tables 1 and 2). An *increase* in the viscosity of plasma (be it natural plasma obtained from patients suffering from macroglobulinaemia, or reconstructed

TABLE 4 Apparent Internal Viscosity of the Red Cell at Different Internal Haemoglobin Concentrations. Data are Calculated from the Viscosities of Suspensions of the Red Cells in Salines of Different Tonicities. Temperature 37 °C, pH 6.6–6.9

Haematocrit %	Relative tonicity	η_r‡	T	p	η_i cp	Internal Hb %
94.1	1	32	0.796	1.9	1.3	33
91.8	1	20.6	0.764	1.5	1.05	33
93.5	1	35.7	0.815	2.2	1.5	33
91.0	1.12	19.6	0.765	1.5	1.05	35
89.0	2	63	0.915	6.0	4.2	48
87.0	2.7	55.5	0.920	6.5	4.55	54
98.0	3.5	380	0.925	7.0	4.9	59
98.0	4	528	0.937	8.5	6.0	61
92.0	0.86	20	0.760	1.5	1.05	30

‡ All viscosity determinations were carried out at shear rates of 160 sec^{-1}

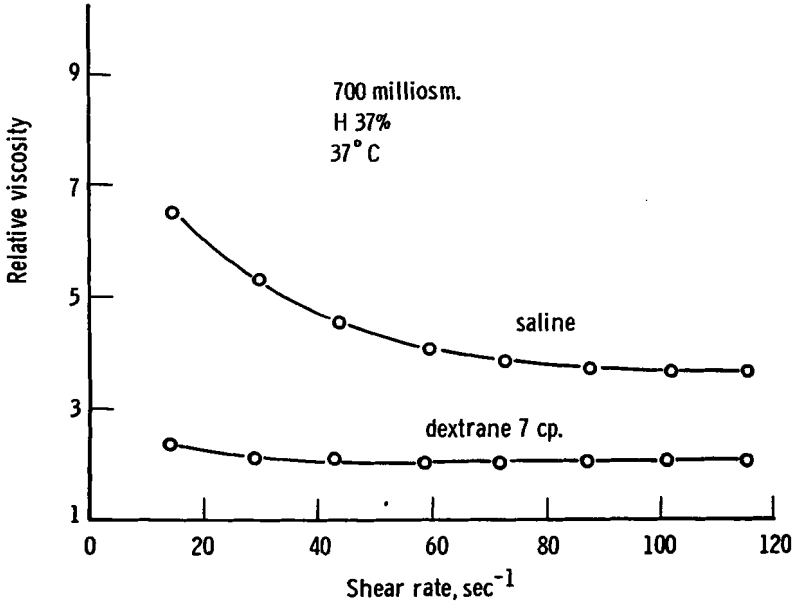


Figure 6. Viscosity of hypertonic suspensions of red cells in saline and in dextrane solutions. Relative viscosity is plotted against the shear rate. Viscosity of the dextrane solution is 7 centipoises. *H:* haematocrit.

plasma in which solutions of macroglobulins and normal proteins were used, or synthetic plasma in which dextrane solutions were employed) results in a *decrease* ofthe relative viscosity of blood. This is directly due to a decrease of p and of T (p being the ratio internal structure of the red cell. The interior of the red cell is of the internal viscosity of the red cell to the viscosity of the continuous phase, and T being Taylor's constant).

As the internal viscosity of the red cells appears to be within the range from 1 to 6 centipoises, some thought could be given to the

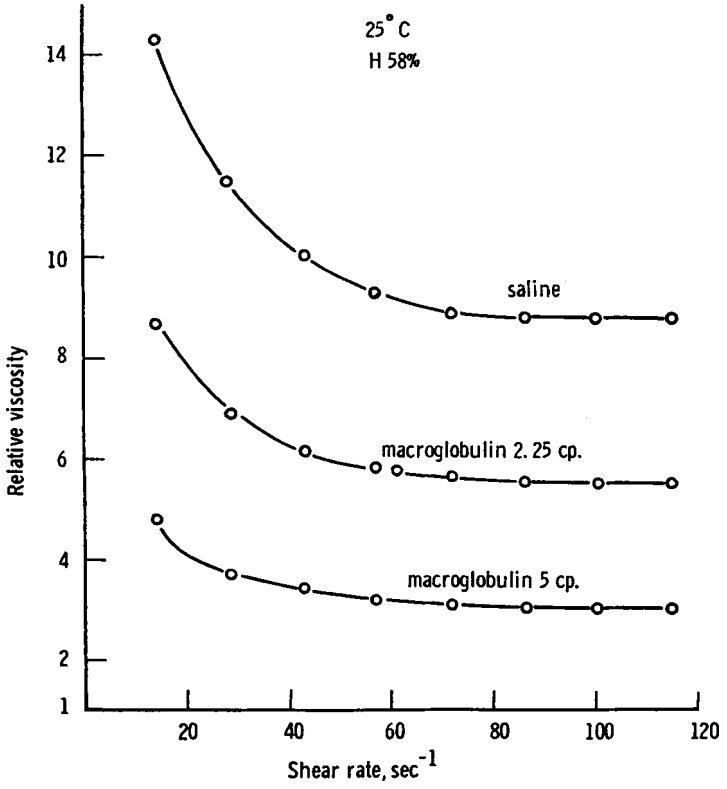


Figure 7. Viscosity of isotonic suspensions of the red cells in saline and in the macroglobulin plasma of 2.25 and 5.0 centipoises. The relative viscosity is plotted as a function of the shear rate. *H:* haematocrit.

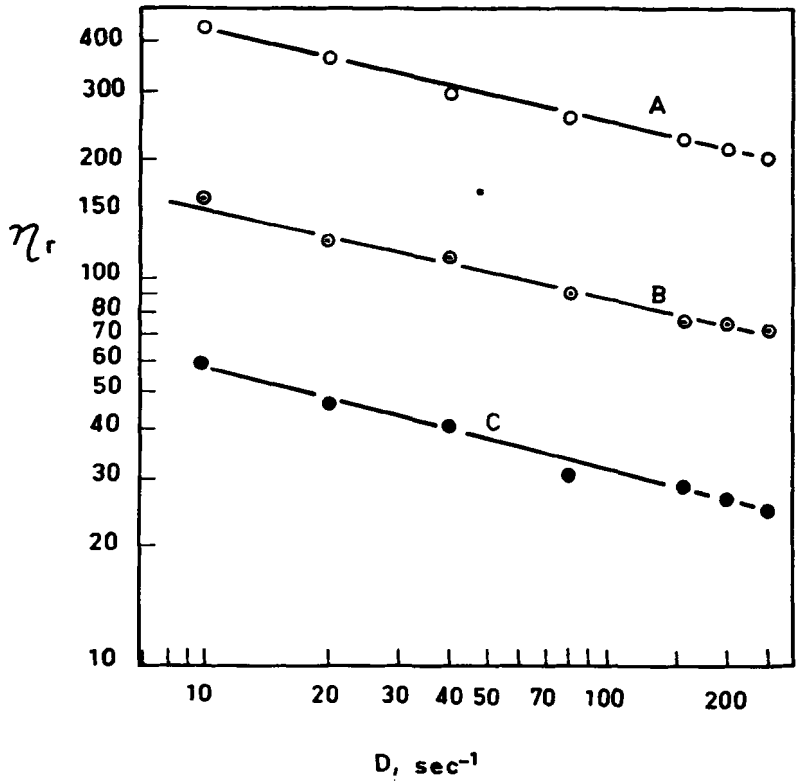
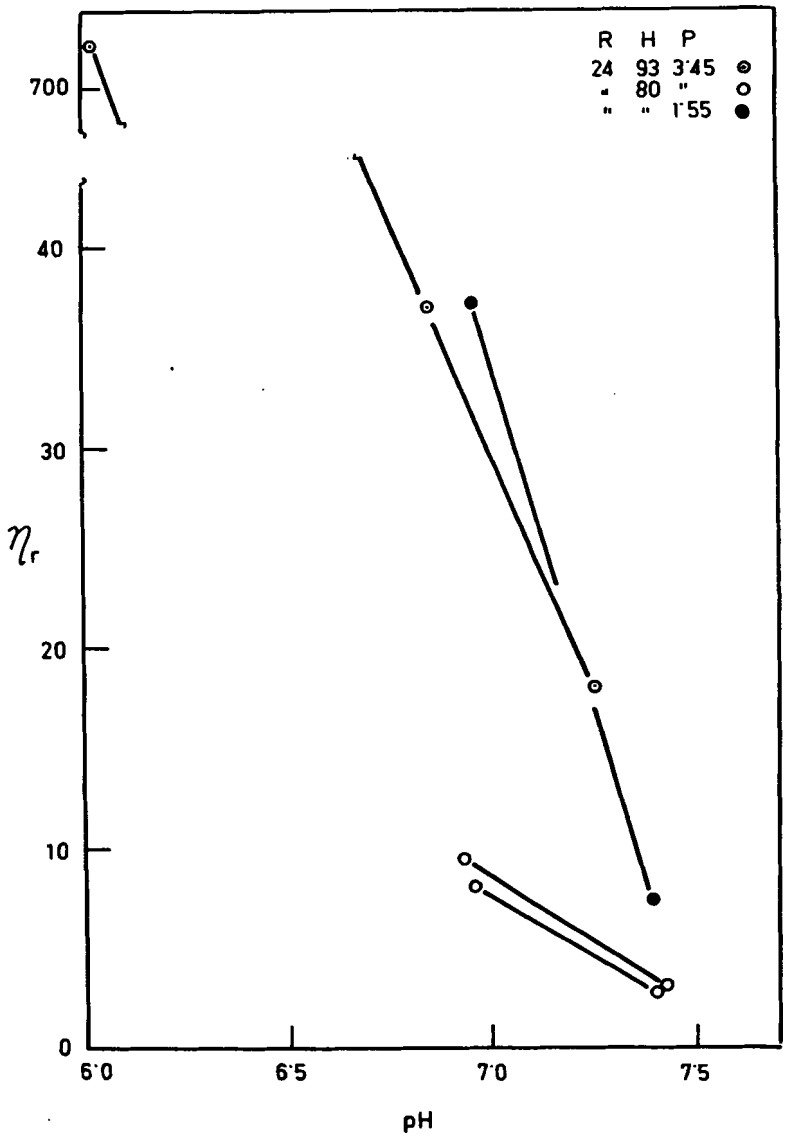


Figure 8. Viscosity of isotonic suspensions of the red cells in the macroglobulin plasmas of different viscosities. All suspensions show 98 per cent haematocrit. The relative viscosity is plotted as a function of the shear rate on log-log scale. *A:* plasma viscosity equal one centipoise; *B:* plasma viscosity equal 2.1 centipoise; *C:* plasma viscosity equal 7 centipoises.

Figure 9. The effect of blood pH on the viscosity of blood flowing through a slit capillary. The relative viscosity, η_r, is plotted against the blood pH. *R:* radius of the channel in microns; *H:* haematocrit, per cent; *P:* pressure gradient in cm H_2O per cm length of the capillary.



fluid although it accommodates usually 33 per cent of haemoglobin of about 64,000 molecular weight. A solution of branched or linear polymer of the same molecular weight would give viscosity from 100,000 to 200,000 centipoises.[31,32,33] Were haemoglobin in true solution, the interior of the red cell would be rigid. Were, however, haemoglobin in a second phase, then any of the usual equations for viscosity of suspensions would supply a similar answer: a viscosity from 1.8-fold to 2.3-fold that of the continuous phase; and this answer corresponds sensibly to the results obtained.

In order to elucidate further this point, it is required to study the viscosity of cells in which the internal concentration of haemoglobin is increased up to 60 per cent, by shrinking the cell in hypertonic solutions of saline or dextrane.

A relationship between the tonicity of the saline and the size of the cell is given, i.e., by LeFevre's equation[34]:

$$V = D + \frac{1 - D}{t} \tag{6}$$

in which V is the cell volume relative to the cell volume at normal tonicity; D is the " dead space " or nonsolvent volume, equal to 0.385; and t is the relative tonicity, the normal (isotonic) tonicity being taken as 1. Haemoglobin concentration would be therefore the normal concentration in a cell under isotonic conditions divided by the relative cell volume V, corresponding to the selected tonicity.

The data for viscosities of suspensions of crenated cells, at different tonicities, and at different levels of haemoglobin con-

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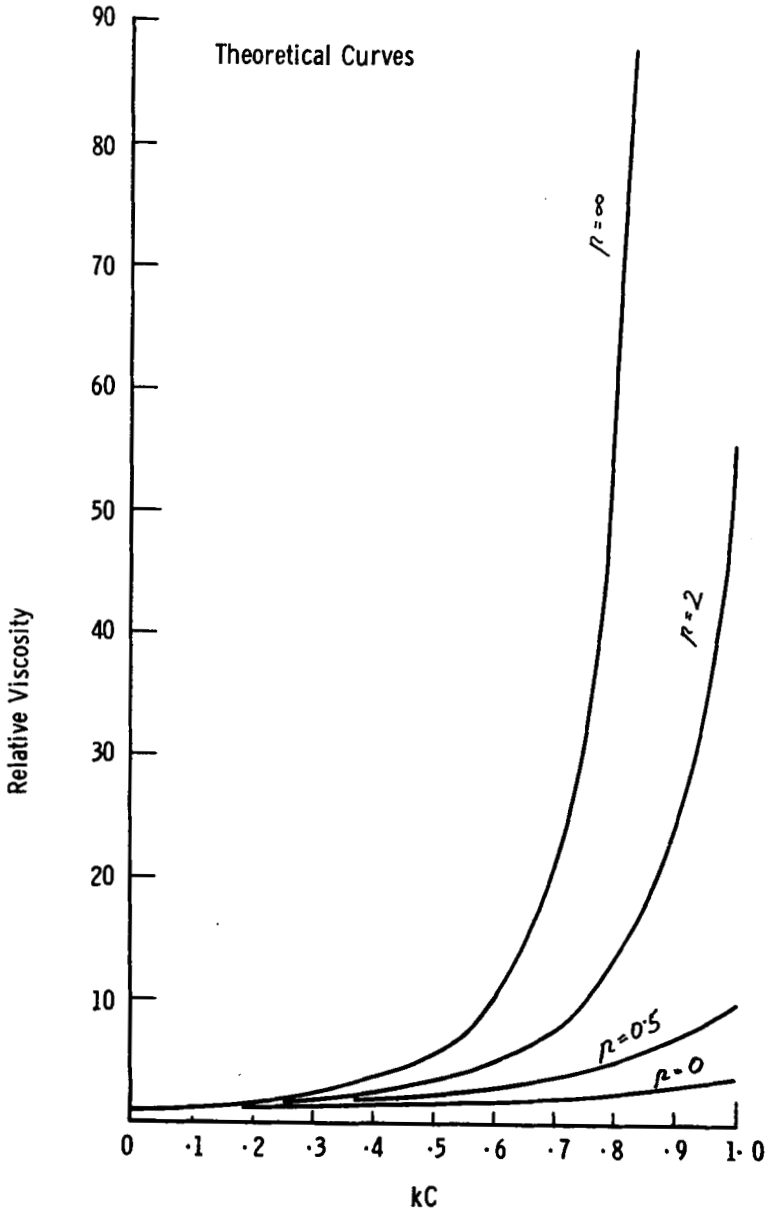
Figure 10 (a). Theoretical curves for the viscosities of monodisperse suspensions of the red cells (or of the fluid drops), drawn from the Eq. (4). The relative viscosity is plotted against the product of k, the packing coefficient, and C, the fractional volume of the disperse phase, for different values of p. " p " is the ratio of the internal viscosity of the drop to the viscosity of the continuous phase.



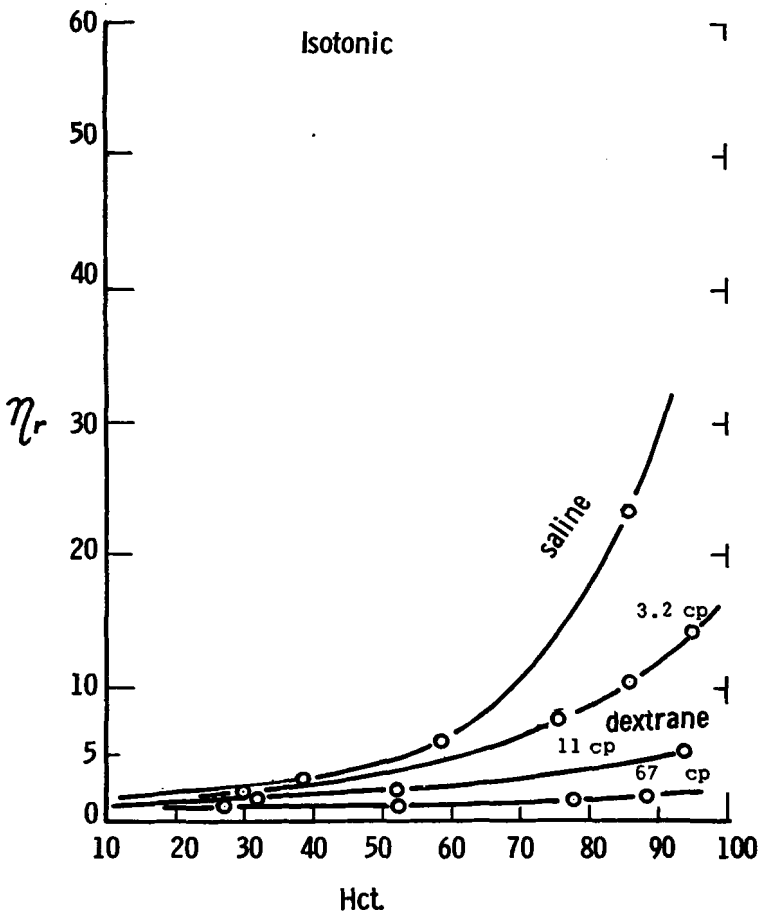


Figure 10 (b). The relative viscosities of suspensions of the red cells in continuous phases of different viscosities. The relative viscosity, η_r, is plotted as a function of haematocrit. Saline and dextrane solutions of 3.2, 11, and 67 centipoises are used. Please note, when comparing with the Fig. 10 (a), that the p values for the normal range of blood should fall between 0.5 and 2; that is, that the apparent internal viscosity of the red cell is between one half to two fold of the viscosity of normal blood plasma. The term normal indicates here the normally encountered blood and includes both the healthy persons and the patients.

centrations in the cells, are given by Table 4. As can be seen, even at the internal concentration of haemoglobin of 61 per cent, the apparent internal viscosity of the red cell is still below 9. This would suggest that haemoglobin molecules are not rigid, and that the interior of the red cell might be also represented as a suspension of fluid particles. Thus, a variation of Eq. (4) could be tentatively employed.

A deduction that haemoglobin is not in true solution but in a second phase, is augmented by observation of Hansen[35] that haemoglobin does not exert any osmotic pressure. Were haemoglobin in true solution, it would exert an osmotic pressure of about 0.5 atmosphere.

The very fact that the red cell is fluid is well known to workers in the field of microcirculation.[36,37,38,39] Such fluidity will be affected by many factors, one of the most common being the blood pH. Effect of blood pH on the viscosity of blood[40,41] and on the apparent internal viscosity of the red cell can be due to a complex influence of the hydrogen ion concentration on the cell size and shape, and on the cell interior and the cell membrane.

The synergistic effect of the haematocrit and of the apparent internal viscosity of the red cell on the relative viscosity of blood can be well visualized when the theoretical or experimental curves are drawn as a function of haematocrit (Figs. 10 (a) and (b)). It would be easy to note that a small variation in the value of p could lead to large changes in the viscosity of blood at higher haematocrits. It must be kept in mind that any changes, due either to p-values or to the apparent internal viscosity of the red cell, observed will be affected by the contributions to viscosity both from the cell interior and from the cell membrane.

2. Rheological Characteristics of the Red Cell Membrane

While the fluidity of the interior of red cell is a necessary condition for the apparent fluidity of the red cell (and thus fluidity of the blood) it is not the unique condition. The second condition depends on the rheology of the membrane, and this has to be elaborated.

Flow properties of a fluid drop depend greatly on the presence or on the absence of an interfacial film or a membrane. In the case when an interfacial film surrounds the drop, the rheological characteristics of this film will influence the transfer of tangential and normal stresses. If this interfacial film is rigid (or elastic—in mathematical sense), then the drop behaves as a rigid particle. But the criteria for the drop fluidity are much more stringent. As already stated, if the internal viscosity of the drop is more than 50-fold larger than the viscosity of the continuous phase, the drop behaves as a rigid particle.

This has been considered by Oldroyd.[17,18] In general, a surface film of very high elasticity will cause the drop to behave as a rigid particle. The effect of purely viscous interfacial film on the viscosity of an emulsion will be the same as that of increasing the internal viscosity of the drop by a factor $\frac{2}{5}(2\phi + 3\delta)/a$, in which a is the radius of the drop, ϕ is the surface shear viscosity, and δ is the surface area viscosity. Boussinesq[19] supplied earlier a similar equation, in which the effect of the presence of viscous interfacial film is equal to that of increasing the internal viscosity of the drop by a term $\frac{2}{3}\delta/a$. If the contribution of the interfacial film to the apparent internal viscosity of a drop (of radius of 2 to 3 μ) is equal to one centipoise, then the surface viscosity of this film should be from 0.000 001 to 0.000 004 surface poises.

While this value for surface viscosity appears to be very low, experimental evidence is actually provided by Nawab and Mason.[42] They found that interfacial films of surface viscosities from zero to about 0.000 008 surface poises enabled the emulsion to retain low viscosity and, thus, each drop behaved as a fluid particle. On the other hand, when the viscosity of the interfacial film increased to 0.000 092 surface poises, the drops became nearly rigid and the emulsion viscosity increased correspondingly. In their experiments, Nawab and Mason used emulsions of drops diameters from 2.5 to 4.5 μ There exists additional evidence that a high interfacial viscosity suppresses the internal circulation in a drop and causes such drop to behave as a rigid particle.[25,43] Schulman and Cockbain[44] observed that the state of the interfacial

film, solid, viscous or gaseous, is reflected in the emulsion which may be semi-solid, highly viscous or very fluid, respectively. Sherman[45] found that, at concentrations of 60 per cent, water-in-oil emulsions may show ten-fold differences in viscosity, depending on the type of emulsifier used. Sherman showed[46] that an increase in the rigidity of the interfacial film might increase hundred-fold the viscosity of emulsions containing droplets comparable in size to the size of red cells.

As the interfacial viscosities will be important to the arguments following, a table of these values, extracted from literature, has been included (Tables 5 and 6). These data[47,48,49,50,51,52,53,54,55] show clearly that surface viscosity of monolayers of proteins is usually between 1 and 4 surface poises; and that very low viscosities, of the order of 0.000 01 surface poise or less is given only by monolayers of very simple and small molecules. And

TABLE 5 Surface Viscosities of Monolayers

Material	Surface viscosity in surface poises		Source
Tricaproin	0.0000065	–0.00036	Joly[47]
Tricaprylin	0.000056	–0.00081	,,
Oleic acid	0.000007	–0.000168	,,
Triolein	0.000031	–0.00014	,,
Palmitic acid	0.00021	–0.00012‡	,,
Stearic acid	0.00116	–0.00026‡	,,
Stearic acid-octadecanol	0.002	–0.05	Boyd and Vaslov[52]
Cetyl alcohol	0.002	–0.0007§	Joly[47]
Sodium lauryl sulfate	0.6	–3.7	Bulas and Kumins[53]
Globulin or insulin	1	–4	Cumper and Alexander[50]
Gluten	1	–4	Tschoegl and Alexander[48]
Pepsin	1	–4	MacRitchie and Alexander[49]
Bovine albumin	1	–4	,,
Poly-epsilon-caproamide	0.04	–0.16	Hotti[54]

‡ Mesomorphic.

§ Non-Newtonian, thixotropic;

Please note that ranges of viscosities, given as the surface viscosity vary with surface area, surface pressure, pH, and ionic strength of the substrate.

even simple molecules, such as saturated fatty acids and alcohols, might show appreciably higher and variable surface viscosities.

Furthermore, the two following questions will have to be considered. First, if an equation relevant to the interfacial film of a fluid drop is valid also for the red cell membrane; and second, what is the meaning of the low surface viscosity and how it can be explained in the terms of molecular structures.

While, usually, the red cell assumes a shape of a biconcave disc (although it can assume a form of a cup, or falling water drop, or a pancake) and the flexibility and deformation of such a disc must influence the flow properties of blood, it should be noted that red cells under hypotonic or hypertonic conditions accept a spherical form. There is no basic difference in the viscosity of such cells, although crenated cells do show an increased apparent internal viscosity. We have to accept that it is the internal viscosity, rather than the shape, which is the more fundamental parameter. Consequently, the equations of Oldroyd[18] and Boussinesq[19] would appear to be applicable.

More difficult is to deduce the molecular structure of the membrane. The membrane contribution to the viscosity of the red cell appears to be between one half of a centipoise to maximum 3 or 4 centipoises, with a mean of 1 to 2 centipoises. A corresponding surface viscosity of the membrane is only of the order of 0.000 001 surface poises. Neither liquid nor condensed monolayers would be thus acceptable. A complex film showing surface thixotropy might be possible. Such films are not unknown.[56,57] According to Joly[47] each type of bulk rheology has a counterpart in the surface rheology. One could accept, in principle, that one of the features of the red cell membrane would be surface thixotropy. However, the continuous existence of the membrane under stresses would indicate that membrane is not a viscous but a visco-elastic body. The problem is to reconcile these considerations and the data of Katchalsky[58] and Rand.[59] Katchalsky, studying haeomolysing red cell, found bulk viscosity of the red cell membrane between 2.7×10^7 and 2.7×10^8 poises, and elasticity of about 5 Kg/cm^2. Katchalsky accepted a Kelvin model, that is, a spring

and dashpot, representing elastic and viscous components, respectively, fixed in a parallel arrangement. Rand, and Rand and Burton,[60] employed a dashpot, or viscous component, in series, and studied haemolysis of the red cell at a variety of membrane tensions. Again, however, Rand's results give viscosity of the membrane in the order of 10^7 to 10^8 (bulk) poises, and elastic modulus (Young's modulus) of the order 0.1 to 10 Kg/cm^2. Rand observed that whenever the area of the cell membrane was made to increase, it became extremely stiff. Time of the test was of importance.

It is not easy to compare properties of surface layers or membranes and properties of bulk systems (see Tables 5 and 6). Although recalculation from one set of units to the other is possible, the physical meaning can be lost. If we would, for instance, try to deduce viscosity of water from the properties of the surface layer of water, we could finish with a material more alike to ice.

Nevertheless, the difference in the data must be taken care of. Is it possible for the membrane to show simultaneously a very low surface viscosity and a very high viscosity? This is quite possible

TABLE 6 Surface Elasticity and Bulk Elasticity of Some Materials

Material	Bulk elastic modulus, Kg/cm^2	Surface elastic modulus, dyn/cm	Source
Casein	44,000	29,000‡	Houwink[69]
Dacron	72,000		,,
10% gelatine gel	0.24	0.16‡	,,
Oxidised trilinolein	ca. 50	ca. 33‡	Elm[72]
Nylon (MW 16000)		0.38–112	Inokuchi[71]
Gluten		1–6	Tschoegl and Alexander[48]
Cytoplasm	0.0001		Crick and Hughes[73]
Crenated red cell	below 5		Ponder[1]
Ghost membrane	10	6‡	Katchalsky *et al.*[58]

‡ Calculated order of values, assuming the thickness of the film to be 66 Å.

Please note that these values are approximate, as elasticity of materials listed is of non-linear type.

if studies are carried out under different conditions. Dilatant systems might show a great difference in viscosity. An analogous to dilatancy non-Hookean elasticity is also feasible. Recently, Mela[61] observed that the modulus of elasticity of sea urchin egg membrane does not follow Hooke's law but is almost directly proportional to the stresses existing at the cell wall. Indeed, Seifriz[62] stated that properties of the red cell membrane change after it has been torn off the cell, the disrupted membrane becoming more rigid. Ponder[63,64] observed that the red cell membrane is non-uniform both in structure and in rigidity, and suggested that washing or treating the membrane, or isolation of the membrane, will change the characteristics of the surface; formation of the fixed framework of ghosts may also modify membrane properties.

Mudd and Mudd[65] found the membrane a plastic or viscous fluid. Some support for the fluidity of the membrane is found in the phenomenon of red cell flicker or membrane fluctuations readily seen in wet preparations of red cell under phase contrast microscopy. This phenomenon apparently does not represent metabolic changes in the membrane or cell interior, but is the result of molecular motions, and can be abolished by increasing the viscosity of the suspending medium.[66] In this respect we must mention work of Curtis[67] who considered in detail the effects of non-Newtonian and thixotropic properties of cell membranes onto the surface undulation, surface wave-forming and adhesion. Curtis suggested that a wave-length of 1 μ corresponds to bulk viscosity of 1410 poises in a cell membrane 70 Å thick. During study of surface viscosities of *Xenopus laevis* cells, Curtis noted[68] that stronger or longer shear may result in a rise of surface viscosity whereas lesser or shorter shear may cause a fall of surface viscosity.

The only possible explanation of the differences between present data on viscosity, and Katachalsky's and Rand's data on viscosity and elasticity is that study of red cell in flow results in the red cell membrane showing very low viscosity, while studies in which a direct attack is made on the red cell membrane, under conditions

of haemolysing cell result in very high viscosities and elasticities. On purely theoretical grounds, an existence of surface dilatancy and surface non-linear (dilatant) elasticity is possible (Fig. 11).

This requires a multiphase thixotropic and dilatant membrane, and the feasible system would appear to be a liquid crystalline layer restrained by two networks of proteins. Such system can show shear thinning and shear hardening, and combination of both; portions of the membrane can become more viscous while others become more fluid or more or less elastic. (Fig. 12).

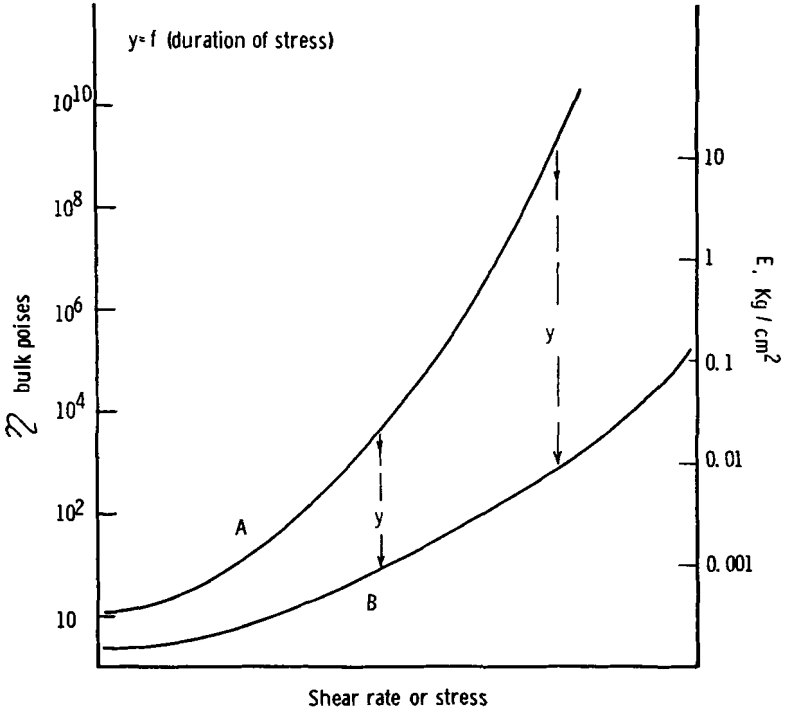


Figure 11. A tentative diagram representing rheology of the red cell membrane. The bulk viscosity and Young's elastic modulus are plotted as a function of the shear rate or stress, and as a function of the duration of such stress. Curves *A* and *B* should represent limits of the rheological values for viscosity and elasticity of the membrane. This diagram attempts to reconcile the present data and the data of other investigators.

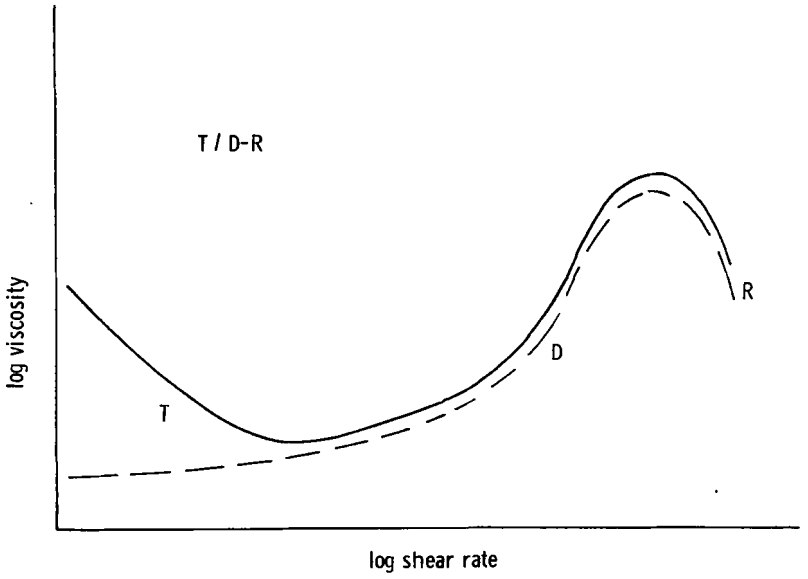


Figure 12. A representation of the red cell membrane as a thixotropic-dilatant fluid with " rupture ". This rupture, R, will correspond to the mechanical breakdown of the membrane due to haemolysis or other causes. The thixotropic phase, T, would be composed of liquid crystals. The dilatant phase, D, would rely mainly on the dynamic protein network which might or might not be an integral part of the liquid crystalline phase.

3. Red Cell Membrane as a Liquid-Crystalline Structure: a Hypothesis

The molecular organization of the red cell membrane was studied and discussed by many workers[74,75,76,77,78,79] but it has been based, mainly, on the studies of ghosts. The direct electron-microscopic tests are not conclusive. Muir,[80] using electron-micrography, observed clear rings containing dark centres which were, in places, packed in a hexagonal pattern. Similar observations were made by Dourmashkin *et.al.*[81] This hexagonal pattern was alleged to be due to the action of saponin and, thus, considered as artefact.[82,83,84] There are, however, indications that the red cell membrane consists of smaller structural units and not from continuous laminae. This was stressed by a number of

investigators.[85,86,87,88,89]. Danielli[90] also agreed that some degree of discontinuity must exist in the laminar " sandwich " of lipids and proteins and introduced " pores " formed by protein lamellae.

The initial tendency to regard the red cell membrane as a bilipid layer, which is not acceptable from the rheological reasons discussed earlier (i.e., such double layer would be much too rigid), moved—independently of any rheological considerations—towards a complex and dynamic membrane. Branton[87] stated that all models, independently if they start with a notion of a bimolecular leaflet or repeating subunits, emphasize the possibility of dynamic interrelations between membrane components and explicitly deny a concept of spatially and temporally uniform membrane. Kavanau[91] regarded the red cell membrane as a dynamic and highly labile entity, shifting between different substructural states with different phases of function, and readily transforming between several equilibrium states.

The present study indicates strongly that the red cell membrane is composed of complex subunits which form a low-viscosity thixotropic phase; and that a second phase, most likely present in a form of a dynamic network of proteins, would be responsible for the dilatancy and/or elasticity.

The liquid phase would be composed of micelles or liquid crystals which structure could be affected not only by the velocity gradient, but also by chemical environment (that is, ionic strength, pH, presence of active compounds, etc.). Although it might appear, at the first sight, that a liquid crystal would not have satisfactory tenacity, it is not necessarily so. Liquid crystals can persist although the molecular components of such crystal can change or interchange.

Brown and Shaw[92] gave an exhaustive review of the field of liquid crystals and directed attention to the work of Bernal[93] who suggested that a liquid crystal, through its own structure, is an organ or protoorgan for mechanical or electrical activity; and through the fact of its orientation, it provides an ideal medium for catalytic action. Through the very fact of being composed of liquid crystals, membrane would be capable of catalytic action

and would respond, in effect, to mechano-chemical and mechano-electrical transformations. Additionally, it has been suggested by Sollner[94,95,96] that any heterogeneity in the structure of an ionic membrane, interposed as it is between two electrolytic solutions of different compositions, will give rise to different potentials and will lead to formation of spontaneous electrical circuits.

The utilization of liquid crystal concept to the red cell membrane has been proposed in 1964 by Dintenfass,[97] on purely rheological grounds. But in the following years, a number of investigators came independently to this idea on purely chemical grounds.[98,99,100] Liquid crystals were found to show selective diffusion of different ions and variable osmotic and permeability properties.[101,102] Lipids,[103] phospholipids,[104] and cholesterol[105,106] containing liquid crystals have been studied.

The rheological characteristics of liquid crystals may greatly vary; they are shear dependent and greatly temperature dependent[107,108,109,110] although our knowledge of rheology of the liquid-crystalline states is far from complete.[111]

Nevertheless, the information available on the rheology of liquid crystals is compatible with the performance of one of the phases of the model suggested. The second phase of the membrane, responsible for the elastic properties, could be due to the existence of a dynamic two-dimensional network of proteins of rather low, and perhaps asymmetric, concentration of proteins on the inner and outer surface of the liquid-crystalline phase. The rheological behavior of this model would be analogous to the thixotropic-dilatant bulk fluid systems.[112,113] In quite an analogous manner, an interlocking of protein molecules and a formation of an elastic network could take place either at sudden or at high stresses.

Small variations of the environment, as for instance the ionic balance, a presence of lytic agents, lipid concentration or lipid type, blood pH, etc., would affect the liquid crystalline structure and might, thus, affect also the rheological characteristic of the membrane.

Heterogeneity of the membrane due to the presence of a number of liquid crystalline units might be responsible for the heterogeneity demonstrated in the surface adsorption of lipids,[114] in the antigen-antibody reactions,[115,116] and in the selectively different structure and composition of the membrane.[117] Heterogeneity of the membrane might solve the apparent contradiction between the views if the red cell surface is polar or hydrophobic, as it is quite feasible that it is both. Containing areas of different affinities, the red cell membrane might have surface properties analogous to the selective polar adsorption patterns demonstrated on solid surfaces.[118,119]

Heterogeneity of the electrical charge might influence the active transport, while fluidity of the membrane might facilitate diffusion.[120] In effect, the modern permeability theories[121,122,123] reconcile well with the new model of the red cell membrane. So are the catalytic and enzymatic processes taking place within the red cell membrane. The latter could be affected, also, by the mechanics of flow through the mechano-chemical transformation principle.[124,125]

Consequent to the rheological studies of the red cell and to the ideas reviewed above, it does appear sensible to consider the red cell membrane as a complex, multiphase, heterogenous and dynamic system. Its rheological structure, as well as its catalytic activity and active transport, could be related, in a tentative manner, to the liquid crystalline structure. While this principle is reasonably clear, the details must remain uncertain.

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