A New Method of Measuring the Deformability of the Red Cell Membrane

By Brian S. Bull and J. Douglas Brailsford

Red cells moving in a stream of fluid can be arrested by means of a network of fibrin threads. Those cells which fold over a fibrin thread present to view a flat strap-like portion of membrane in which the strain can be easily observed and accurately measured both optically and by scanning electron microscopy. Preliminary results obtained by this method show elastic strains of nearly 300% and indicate that the stress required to produce these large strains is an order of magnitude greater than that reported by other methods.

Measurements of the physical characteristics of the red cell membrane are a prerequisite to the formulation of any membrane model which attempts to duplicate those physical attributes. A membrane model which is successful in duplicating such specific physical characteristics of the red cell as its marked deformability, elasticity, incompressibility, and conservation of surface area, would significantly narrow and clarify discussions of red cell membrane ultrastructure. Any proposed arrangement of membrane macromolecules would have a lengthy list of physical characteristics to explain, in addition to the many biologic functions which the red blood cell membrane is already known to subserve.

Determining the physical characteristics of a red cell membrane poses formidable technical problems. The measurements must be made on wet membranes, for drying is known to alter drastically membrane characteristics. For practical purposes this limits observations to light microscopy of red cells suspended in isotonic diluent. The red cell must then be deformed by a measurable and controllable force which, in turn, requires some method of holding the cell during the deformation. The means chosen to arrest the cell and the method by which the deformation is produced ideally should be atraumatic to red blood cell membranes and should permit clear visual observation. A gradually varying series of forces can then be applied and the resultant deformation measured optically. Even so, the experiments will strain the limits of light microscopy, since the dimensions of the red cell are so small relative to the wavelength of visible light.

The earliest comprehensive estimate of the physical characteristics of the red blood cell was made by Rand using the micropipette technique of Mitchison and Swann. In this method, a micropipette is used both to immobilize the cell and to deform the membrane. A known negative pressure is used to aspirate a...
hemispheric portion of the red cell into the pipette. Increasing the negative pressure causes the membrane to move further into the pipette and to form a cylindrical tongue with a hemispheric end. The method has received considerable attention for the measurement of red cell deformability by La Celle and Leblond and has recently been developed mathematically by Evans, who has shown that large deformations are involved in producing the "tongue" lengths observed. The method has several attractive features: Only easily measured static pressures are involved, the deformation is axially symmetrical, and the deformed portion under observation is virtually isolated from the rest of the membrane material. Disadvantages, however, are: (1) The membrane material is in close contact with the pipette wall which, in the case of glass, is known to interact with the red cell membrane. (2) A pressure difference is established across the membrane itself. This is a nonphysiologic condition, and its effect is not known. (3) The deforming force is applied to a small cross section of the material inside the pipette, while the maximum deformation occurs at the tip of the pipette. This ultimately leads to instability (necking down) which limits the observable range of deformation. (4) It is not possible by this method to observe the dynamic behavior of the cells under conditions resembling those which obtain in the blood circulation.

A method which largely avoids the interfering effects of glass has been described by Hochmuth and Mohandas and Hochmuth et al. Measurements are made on "tethered" cells; that is, cells are attached to a glass slide at one or two points only and deformed by fluid shear. The absence of the constraint imposed by the wall of a pipette makes it possible to produce extremely large deformations by this method. Interpretation of the results is rendered somewhat uncertain, however, by the complex shape of the red cell and the markedly non-uniform distribution of stress.

Here we describe a third method, the examination of red cells arrested by being folded over fibrin strands and deformed by continuous fluid flow. As in the case of tethered cells, the whole process can be carried out in an optical chamber incorporated in a microscope slide. It can therefore be observed either by phase-contrast or by interference microscopy. Cells deformed in this way can quite easily be fixed in the deformed state while still under the microscope. They can then be prepared for scanning electron microscopy, thus permitting precise confirmation of the optical measurements. Under these circumstances, the red cell does not contact any foreign surface, and it is subject to stresses which are qualitatively similar to those encountered in the normal circulation. It is possible, moreover, to subject cells to the full range of mechanical stresses which they are capable of withstanding.

MATERIALS AND METHODS

Principle

Red cells in a moving stream of fluid can be arrested by stationary fibrin strands. When this occurs in the manner shown in Fig. 1, each cell forms two roughly spherical lobes which blend into a flat straplike region where the membrane material actually passes over the strand. The deformation results from fluid viscous drag forces which act on the red cell membrane and equilibrate with the restraining forces produced by the fibrin strand.
For at least two reasons this particular type of deformation is unusually amenable to analysis:

1. The flat straplike region where the cell bends over the fibrin strand can be seen clearly and its width measured. The strap here consists of a double layer of membrane so that the cross-sectional area of membrane material on which the deforming forces are acting can easily be determined. 

2. The cells slide freely along the fibrin strand during the formation of constellations like those in Fig. 2. This implies that, initially at least, the membrane is not stuck to the strand; and, therefore, the deforming forces must give rise to almost pure tension in the straplike portion of membrane where it approaches the fibrin strand.

**Techniques**

Red cells in EDTA-anticoagulated whole blood were obtained from healthy human donors. The cells suspended in autologous plasma were allowed to interact with fibrin strands in a flow chamber previously described. After the “washing on a line” aggregates had formed, a fixative solution of 2.5% glutaraldehyde in isotonic saline was substituted for the red cell suspension and a constant flow rate maintained for 5 min until complete fixation had been achieved. Before fixation, the flow velocity of the blood cell suspension in the region immediately adjacent to the red cell clump was measured by determining the velocity of nearby unattached red blood cells. This was done by

**Fig. 1. Stages in the formation and release of an aggregate of red cells arrested by becoming folded over a fibrin strand.**

**Fig. 2. Scanning electron microscope picture of an aggregate of red blood cells arrested by a fibrin strand. × 5000 (reduced).**
taking multiple-exposure flash pictures in rapid sequences under the optical microscope. The un-attached red blood cells in the vicinity show up as multiple images. From these images the velocity of flow can be determined, since the time interval between the flashes is accurately known. In the first experiments, double images were used. Triple images of <10^{-3} sec duration, however, were found to be superior, as there was then little occasion for ambiguity in interpreting the images.

After fixation, the specimen was prepared for scanning electron microscopy by critical-point drying and gold plating. The specimen was examined in an AMR-1000 scanning electron microscope operating in the secondary emission mode.

RESULTS

The red cell membrane is known to be substantially incompressible in two dimensions for weak forces,\(^9\) that is, the surface area of the membrane remains constant. As a result, the elongation of the membrane in a direction at right angles to the fibrin strand must be equal to the reduction in width of the membrane in a direction parallel to the strand. The original unstressed width of the membrane material is known from the meridional circumference of the undeformed cell, and, since the final distorted width can be measured, the local membrane strain is known. Thus, by relating the cross-sectional circumference of the distorted membrane where it crosses the fibrin strand to the original circumference of the cell membrane in the corresponding plane, the extent of the distortion in any particular cell can be calculated. This has been done on the cells shown in Fig. 2 which, for the purposes of clarification and identification, have been redrawn in outline form in Fig. 3. The calculations on cell 2 are illustrative. According to Evans and Fung,\(^9\) a normal cell of diameter 7.8 \times 10^{-4} \text{ cm} at 300 milliosmols osmotic tension would have a surface area of 135 \times 10^{-8} \text{ sq cm}. Cell 2 is from a population in which the mean diameter was found to be 6.3 \times 10^{-4} \text{ cm}. The surface area can, therefore, be taken to be 88 \times 10^{-8} \text{ sq cm}. A spherical cell of the same surface area would have a diameter of 5.3 \times 10^{-4} \text{ cm} and a circumference of 16.6 \times 10^{-4} \text{ cm}. The circumference of the slightly offset plane section of this sphere, corresponding to the position of the
fold in the distorted cell, can be calculated, and is found to be $16.3 \times 10^{-4}$ cm. Since the measured width of the membrane material where it crosses the fibrin strand is $3.36 \times 10^{-4}$ cm, the elongation ratio $\lambda$ for this cell is $16.3$ divided by $2 \times 3.36$, which equals $2.42$.

Cells 1–6, treated in a similar fashion, show the following extension ratios: Cell 1, 2.47; cell 2, 2.42; cell 3, 2.83; cell 4, 2.81; cell 5, 2.57; cell 6, 2.94.

The Lagrangian strain $\epsilon$ is related to the elongation ratio $\lambda$ by the equation:

$$\epsilon = \frac{\lambda^2 - 1}{2}$$

Substituting the experimental value of $\lambda$ obtained for cell 2 in this equation gives the value of the strain $\epsilon = 2.43$.

At the velocities studied, the fluid flow round the cells is always laminar because both the depth of the optical chamber and the dimensions of the red cell are so small. Stoke’s Law therefore applies. This law states that the viscous drag on a spherical body of radius $a$ in a fluid of viscosity $\mu$ is equal to $6\pi a \mu U$, where $U$ is the velocity of the fluid relative to the body.

When the red cell first hits the fibrin strand, it presents to the flow a circular disc profile. A disc has the same drag resistance as a sphere of equal radius, but in this case the resistance is distributed between the two portions on either side of the restraining strand. The value of $a$ in Stoke’s formula to obtain the drag on each lobe of a symmetrically disposed red cell is, therefore, half the radius of the discoid cell.

As the cell distorts, the drag will change. Limits to the possible variation can be found by considering the possible shapes the cell may assume: (1) Two equal spherical lobes, each of half the total surface area of the cell membrane; (2) two equal spherical lobes, each of half the total volume of the cell; and (3) two equal hemispheric lobes, each of half the total volume of the cell and lying with their flat faces adjacent. These faces could not then contribute to the viscous drag.

The drag force in each of these cases relative to that on a sphere of half the radius of the discoid cell would be (1) 1.18, (2) 1.15, and (3) 0.84. The reason for the small variation is that the drag force is proportional to the first power of the radius, whereas the surface area and volume are proportional to the second and third powers, respectively. The effect of asymmetrical disposition of the cell with respect to the fibrin strand can be corrected for because the distorted shape of the cell is known.

Not only is the extreme variation represented by the above ratios small, but the conformations (1) and (3) are physically unattainable in a normal red cell, owing to the restriction imposed by the relation between its surface area and volume. For cell 2, the diameter of the discoid cell was $6.3 \times 10^{-4}$ cm. The velocity of flow measured by flash photography was $3.2 \pm 0.2$ cm/sec. Substituting these values in Stoke’s formula gives a viscous drag on each side of the cell of $0.95 \times 10^{-4}$ dynes. The width over which this force is distributed in the strap-like region of the cell is $2 \times 3.36 \times 10^{-4}$ cm. Therefore, the tension in the membrane, expressed as force per unit length, is 0.14 dynes/cm. If the membrane is assumed to be $10^{-4}$ cm thick, the stress in the material when elongated to 2.4 times its original length is $1.4 \times 10^5$ dynes/sq cm.
DISCUSSION

It is likely that the values of extension for cells 3 and 4 are overestimated, since the membrane is wrinkled and therefore shortened as it crosses the fibrin strand. The high value obtained on cell 6 is probably due to its asymmetrical disposition over the fibrin strand. Cells 1, 2, 5, and 6, although they show irregularities due to unevenness of the underlying fibrin, contain no wrinkles of sufficient size to affect the measurements seriously.

The membrane material in red cells, such as those illustrated in Fig. 2, has been shown to be extended to nearly three times its original length in the straplike region, yet the cells immediately resume a symmetrical biconcave shape on leaving the fibrin strand. This indicates that the red cell membrane can be elastically deformed at least 300%.

The deformation in the straplike region of the cells shown in Fig. 2 is produced by a membrane tension of approximately $140 \times 10^{-4}$ dynes/cm. A similar deformation in pipette experiments was reported by Evans to be produced by a membrane tension of $14 \times 10^{-3}$ dynes/cm. The discrepancy is too large to be accounted for by experimental error, and its cause has not yet been identified. The method, however, opens up two interesting possibilities for further experiment. First, the applied stress can be carried into a region of higher values than are attainable by the pipette method. Second, it is possible to make direct observations on both the contraction of the straplike region and the corresponding elongation in the orthogonal direction. This should give direct evidence of the validity of the assumption that the surface area is invariant under the measured conditions of stress.

REFERENCES

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