MEASUREMENT OF THE ELASTIC MODULUS FOR RED CELL MEMBRANE USING A FLUID MECHANICAL TECHNIQUE

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ABSTRACT Red cells which adhere to a surface in a parallel plate flow channel are stretched when acted on by a fluid shear stress. Three types of stretching are studied: whole cell stretching, the stretching of a red cell evagination, and tether (long, thin membrane process) stretching. In addition, the stretching of a large scale model cell attached to a surface is studied in a Couette flow channel. The results indicate that the uniaxial stretching of red cell membrane can be described by a linear stress-strain relationship. Simple theories developed from free body diagrams permit the calculation of a value for the modulus of elasticity of cell membrane in each of the three experiments. In all cases the value for the modulus is on the order of 10^4 dyn/cm^2 for an assumed membrane thickness of 0.01 μm. It was also observed that red cell tethers steadily increase in length when the fluid shear stress is greater than approximately 1.5 dyn/cm^2 and tether lengths in excess of 200 μm have been achieved. Tethers appear to possess both fluid and elastic properties.

INTRODUCTION

In order to characterize the elasticity of a material, a well-defined force per unit area (stress) must be applied to the material and the resulting deformation (strain) must be measured. In linear elasticity the modulus of elasticity is defined as the slope of the stress-strain curve. At the cellular level difficulty is encountered in determining the stress-strain curve for (say) the red cell membrane since the forces and deformations involved will be quite small. For example, assume for convenience that the cell membrane has a value for its modulus of elasticity equal to that for latex rubber (10^7 dyn/cm^2), and assume that upon application of an external force the membrane experiences an overall strain of 100%. If a linear stress-strain relationship is valid, then the *stress* in the membrane will be 10^7 dyn/cm^2. However, the *force* involved will be equal to the stress *times* the membrane cross-sectional

area. For a cell membrane which is, for example, 5 μm wide and 0.01 μm thick (giving a cross-sectional area of 0.05 μm^2), the force required to produce the membrane stress of 10^7 dyn/cm^2 is only 5×10^{-4} dyn. Smaller assumed values for the elastic modulus would give even smaller values for the force required to produce a given strain.

We have resorted to a fluid mechanical technique in order to produce small forces on the order of 10^{-4} dyn or less. When fluid flows over a solid surface, the fluid in contact with the surface has a velocity of zero (the "no-slip condition"). The fluid, however, exerts a finite shear stress on the solid surface. This stress can be varied over a wide range of values simply by varying the rate of fluid flow. For a red cell which adheres to a solid surface, the stress which acts over the surface of the cell will be approximately the same as the stress which acts on the solid surface in the absence of the cell since the cell is a relatively flat body which will cause only minimum disturbance to the flow. A shear stress of 10 dyn/cm^2 (note that the maximum stress at the wall of an artery is on the order of 100 dyn/cm^2) will produce a net force of 5×10^{-6} dyn on a red cell with an area of 50 μm^2 in contact with the flowing fluid.

Other techniques have been used in experiments designed to determine a modulus of elasticity for red cell membrane. Rand and Burton (1) measured the pressure required to suck a portion of a red cell into a micropipette. They then used the "law of Laplace" to calculate the lineal tension (force per unit length) in the membrane at a given pressure. However, Fung and Tong (2) have noted that the law of Laplace is not valid in the Rand-Burton experiment since the tension is not uniform throughout the membrane. (This is especially obvious at the mouth of the micropipette.) Nevertheless, Rand (3) used the law of Laplace to obtain a value between 7×10^6 and 3×10^8 dyn/cm^2 for the elastic modulus of red cell membrane at rupture based upon an estimated "critical strain" (i.e., the strain at rupture) between 8 and 42% and a membrane thickness between 0.01 and 0.1 μm. Another estimate of 2.4×10^7 dyn/cm^2 for the elastic modulus of red cell membrane was obtained by Katchalsky et al. (4) for an assumed membrane thickness of 0.006 μm. Their calculation was based upon an experimentally determined value for the change in volume of a red cell as it swells as a sphere before rupture and an estimate of the pressure within the sphered cell at rupture. Although the investigations of Rand and Katchalsky et al. differ in experimental technique, they are similar in that the elastic moduli they obtained were at high stresses and were of the same order of magnitude (10^7 dyn/cm^2).

EXPERIMENT

A parallel plate flow channel (Fig. 1) is used to exert a shear stress on fresh human red cells which have been allowed to sediment and subsequently adhere to a clean glass cover slip coated with bovine serum albumin (BSA). Red cells are prepared by washing them twice in phosphate-buffered saline (PBS) *without* protein and resuspending them in PBS at an approximate hematocrit of 0.5%. In the flow channel, a fluid (PBS with 0.1% BSA and 1–2% native plasma at pH 7.4 and osmolarity $\simeq$ 260 mosM) flows between the glass cover slip

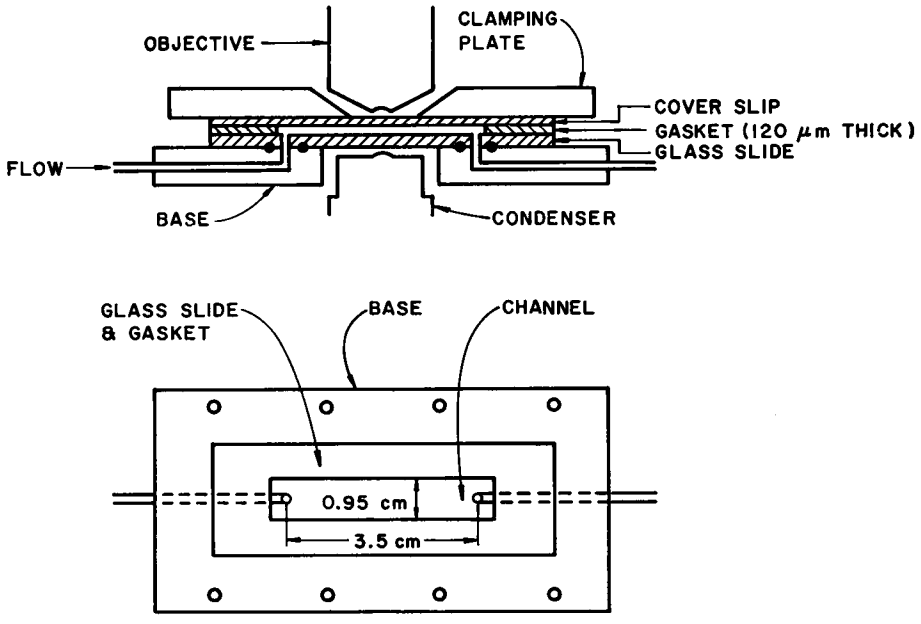

FIGURE 1 Flow channel.

and a glass slide through inlet and outlet holes in the slide. The slide and cover slip are separated by a Parafilm gasket (American Can Company, Neenah, Wis.) approximately 120 μm thick (the exact thickness is measured with a micrometer) and which is cut to form a channel 0.95 cm wide and 3.5 cm long. The slide-gasket-cover slip sandwich is held between a base plate and a clamping plate. The rate of flow is controlled by means of a Harvard variable speed DC infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.). The deformation and stretching as a function of flow rate of individual red cells adhering to a surface is recorded on 16 mm film with a Bolex camera (Paillard S. A., Sainte Croix, Switzerland) coupled to a Leitz microscope with a dry phase-contrast objective (E. Leitz, Inc., Rockleigh, N. J.). No eyepiece or camera lens was used. At a given volume flow rate Q (determined by weighing the amount of saline which flows through the flow channel in a given time period), the shear stress at the surface τ_s is given by

$$\tau_s = 6\,\mu Q/Wh^2, \qquad (1)$$

where μ is the fluid viscosity (approximately 0.01 P), W is the channel width, and h is the thickness of the Parafilm gasket. All experiments were performed at room temperature (25°C).

Whole Cell Stretching

The stretching of whole red cells in the flow channel has been discussed previously by Mohandas (5) and Hochmuth and Mohandas (6). The work is included here for the sake of convenience and completeness. A stretched red cell is shown in Fig. 2 *a*. In any one experiment the duration of flow at a given rate was less than 30 s. At the end of this period the flow was

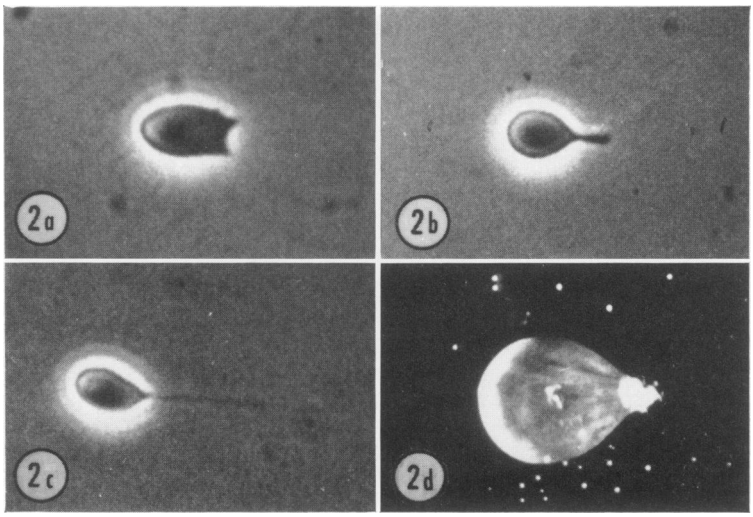

FIGURE 2 Photomicrographs and photograph made from 16 mm film of the cell stretching experiments: (*a*) whole cell, (*b*) evagination, (*c*) tether, (*d*) model cell. Flow is from right to left.

stopped, the cells were allowed to return to their unstressed state, and then a different flow rate (shear stress) was imposed. Both the change in shape and the overall elongation as recorded on 16 mm film were analyzed with a stop-action projector.

Stretching of a Red Cell Evagination

In *one* experiment a single red cell with an evagination was discovered (Fig. 2 *b*). Under conditions of flow the cell appeared to be attached to the albumin-coated glass surface *only* at the upstream tip of the evagination as shown in Fig. 2 *b*. An increase in flow caused the evagination to increase in length. The change in length and the diameter of the evagination were measured with a filar micrometer eyepiece.

Tether Stretching

Blackshear (7) and Hochmuth et al. (8) have observed that during cell detachment long membrane processes, called "tethers" by Blackshear, can be formed. Fig. 2 *c* shows a detached red cell which is anchored to the surface by a tether. The tether usually adheres to the surface only at its end point. To form a tether, a suspension of 0.2% by volume red blood cells in PBS is allowed to settle on a BSA-coated glass surface. As mentioned above, flow is initiated with a solution of 0.1% BSA in PBS with 1–2% native plasma. The plasma prevents a tethered cell from readhering. (Cells stick readily to a BSA-coated glass surface but very few cells will adhere to a glass surface which has been exposed to plasma or fibrinogen [8].) On the BSA-coated glass surface, a red cell will usually adhere at two points at its leading edge (Fig 2 *a*). Pulsing the flow usually causes one of the points of attachment to be pulled free. The tether is then formed at the remaining attachment point by imposing a steady shear stress on the cell in excess of approximately 1.5 dyn/cm^2. For a tether with an initial length l_0, the flow is started and l, the tether length at a given shear stress, is measured with a filar microm-

eter at 30, 60, and 90 s. The readings at these three times are averaged arithmetically. At a "moderate" shear stress (approximately 1.5 dyn/cm^2) these three readings are within a few percent of each other. The flow is then stopped and l_0 (the point at which the tether begins to experience brownian motion) is measured again.

Model Cell Stretching

Experiments on the deformation of a large scale model cell which was glued with silicone rubber to a solid boundary (see Fig. 2 *d*) were performed using a Couette flow channel. The Couette flow field is produced by a 6 inch wide timing belt running on two pulleys inside a tank. The distance between the axes of the drive and idler pulley of the belt is 31 cm and the outer diameter of a belt as it wraps around a pulley is about 9 cm. The gap between the belt and the parallel wall were the model cell is mounted is 5.0 cm. A silicone oil with a viscosity of 567 P at room temperature was used in the experiment reported here. With the oil in the tank, the shear rate between the belt and the wall could be continuously varied from approximately 0.7 to 4 s^{-1} and, thus, the shear stress varied from 400 to 2,270 dyn/cm^2. The model cell consists of a thin 4 cm diameter latex rubber envelope molded into a biconcave shape and filled with silicone oil. The fabrication of model cells of this type is described in a paper by Sutera et al. (9). The deformation and elongation of the model cell (Fig. 2 *d*) as a function of shear rate (belt speed) is recorded on 16 mm film and analyzed with a stop-action projector.

RESULTS

The elongation of whole red blood cells when acted on by a fluid shear stress τ_s (see Eq. 1) is shown in Fig. 3. The extension ratio λ is defined as the length of the

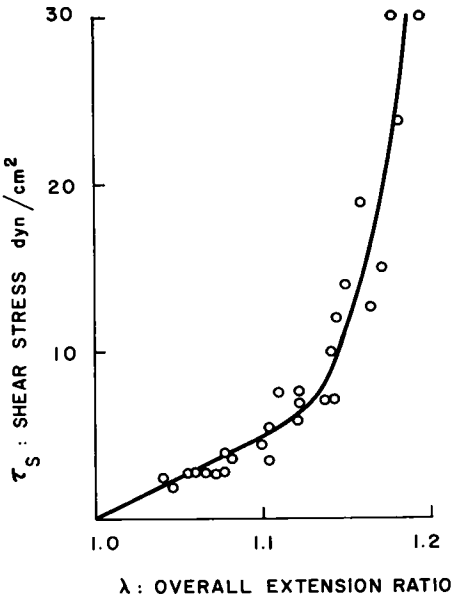

FIGURE 3 The variation of extension ratio with fluid shear stress at the surface for whole cell stretching.

cell at a given τ_s divided by the length of the cell when $\tau_s = 0$ ($\lambda = l/l_0$). This figure presents data from 10 cells with approximately the same "width" of attachment at the upstream, leading edge of the cell of 2–3 μm. Smaller widths of attachment will produce greater extension ratios at a given fluid shear stress. The data in Fig. 3 indicate a linear behavior when the overall increase in cell length is less than approximately 13% of the original length. This represents an increase in length of 1.1 μm for a red cell with an undeformed diameter of 8.5 μm.

The data showing the strains [change in length divided by original length, $\epsilon = (l - l_0)/l_0$] undergone by the red cell evagination are presented in Fig. 4. In all, 25 data points were obtained before the evaginated red blood cell detached from the surface. The straight line in Fig. 4 represents a least squares fit with respect to the abscissa (strain axis). Note that the strain in the evagination was as high as 80% at a shear stress of 2.6 dyn/cm^2.

A total of 217 stress–strain data points for tethers were obtained for 37 different red cells from 13 different donors. A least squares fit with respect to the abscissa plus and minus the probable error (the 50% confidence limits) is shown in Fig. 5. For the sake of neatness the 217 data points are not plotted. The expression for the straight lines in Fig. 5 is

$$\tau_s/\epsilon = 1.31 \pm 0.38 \text{ (dyn/cm}^2\text{)}.$$

In the tether stress-strain experiments, the tether lengths at zero shear stress varied between 2.5 and 24 μm. A tether is capable of undergoing a large increase in length. At a shear stress of 3.5 dyn/cm^2, tether strains as high as 4 (representing an increase in length of 400%) were measured (Fig. 5). Photomicrographs made from 16 mm film which illustrate changes in length of a given tether are shown in Fig. 6.

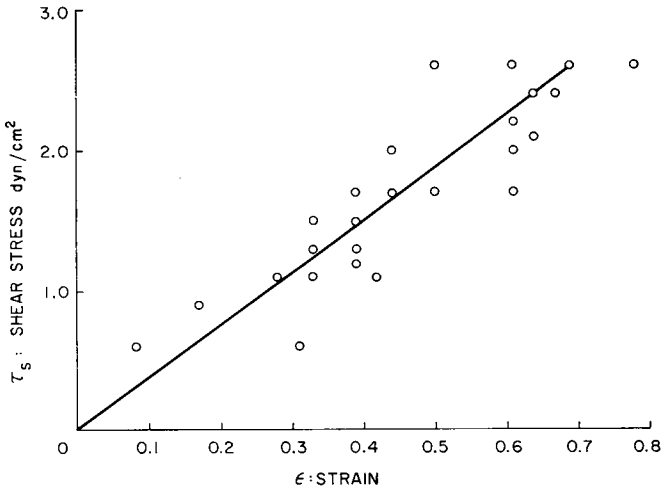

FIGURE 4 The (fluid shear) stress-strain data for a red cell evagination.

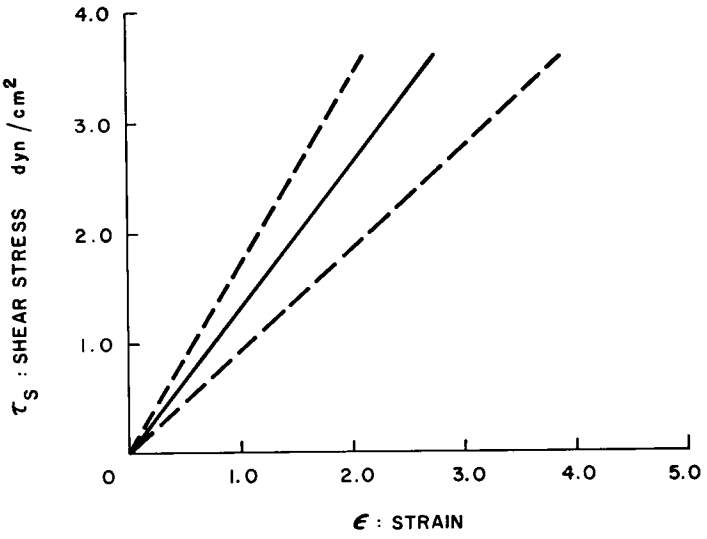

FIGURE 5 The (fluid shear) stress-strain curves for red cell tethers. The solid line indicates a least squares fit with respect to the strain axis and the two dashed lines indicate the probable error (50% confidence limits).

In this figure one cell is tethered to another cell so that the beginning and end of the tether is obvious. The diameter of the tether shown in Fig. 6 *a* is probably right near the limit of optical resolution (0.25 μm). Examination of scanning electron micrographs of tethers indicate values for the diameter which are on the order of 0.1–0.2 μm. The diameter of a tether appears to decrease when it is stretched. An unstressed tether appears relatively "fat" while a highly stressed tether will disappear when viewed with the optical microscope and then reappear when the shear stress is decreased. Often when a tether is stretched suddenly, little "globules" appear in the tether as shown in Fig. 6 *b*. These globules can slowly work their way up the tether and then disappear into the red cell. It is suggested that the globules consist of hemoglobin which is trapped in the tether when the tether suddenly stretches. If the tether is a hollow cylinder of membrane material with hemoglobin on the inside and if the thickness and volume of the membrane material remain constant when the tether is *suddenly* stretched, then the internal volume of the tether would decrease during stretching.[1] The excess hemoglobin in the tether which does not rapidly escape into the red cell might tend to "ball up."

In addition to rapidly elongating when subjected to a sudden increase in fluid shear stress, the tether length would gradually increase with time when the tethered

[1] Let V_t equal the volume of the tether material and let V_i, D_i, t, and l equal the internal volume, internal diameter, thickness, and length of the tether. Then $V_t = \pi D_i t l$ ($t \ll D_i$) and $V_i = \pi D_i^2 l/4$. The elimination of D_i between these two equations gives $V_i = V_t^2/4\pi t^2 l$. Thus, as l increases V_i decreases if V_t and t remain constant.

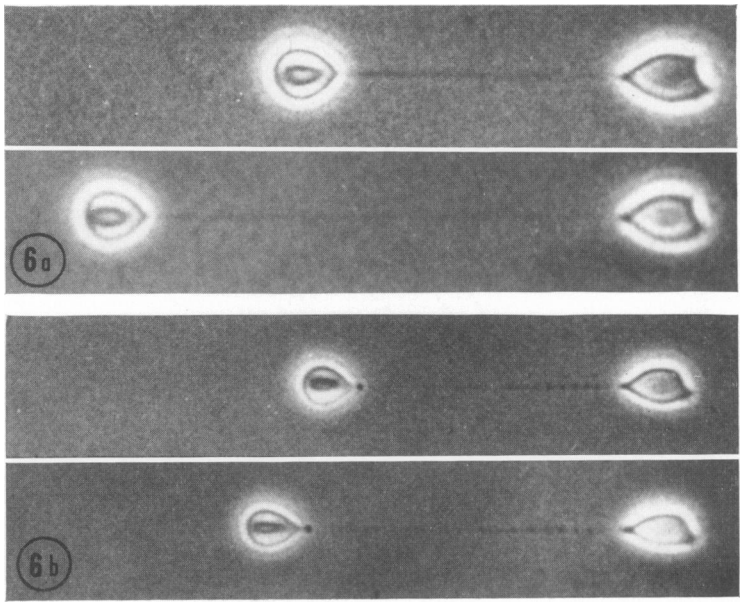

FIGURE 6 Photomicrographs made from 16 mm film of red cell tethers: (*a*) normal tethers, (*b*) tethers with "hemoglobin globules." Flow is from right to left.

red cell was subjected to a constant shear stress in excess of approximately 1.5 dyn/cm^2. The curves in Fig. 7 show a tether growing from essentially a zero length to a length of approximately 70 μm in 300 s for a cell subjected to a constant fluid shear stress of 3 dyn/cm^2 (the shear stress and tether length are shown in the upper and lower graphs, respectively). At the 300 s mark the shear stress was decreased in such a way as to hold the tether length constant. Note that after 500 s (at the 800 s mark) the shear stress is constant and equal to approximately 1.5 dyn/cm^2. This shear stress can be called "the critical stress for tethering." A shear stress in excess of this value will cause the formation of a tether and will cause the tether length to increase steadily with time while a shear stress which is less than this value will cause the tether length to decrease steadily with time until the tether is reabsorbed by the red cell. Fig. 8 shows two constant tether length experiments performed on a single cell and illustrates that when the shear stress is less than the critical stress for tethering, the shear stress must be increased in order to hold the tether length constant and vice versa.

Data similar to those shown in Figs. 7 and 8 have been obtained for 13 additional red blood cells. For a given shear stress in excess of the critical stress for tethering it was observed that the rate of growth of a tether tends to increase as the constant shear stress is increased. However, it should be noted that at a given shear stress, the tether growth rate for different red cells could vary by as much as a factor of three and that the critical stress for tethering varied from 1.3 to 1.75

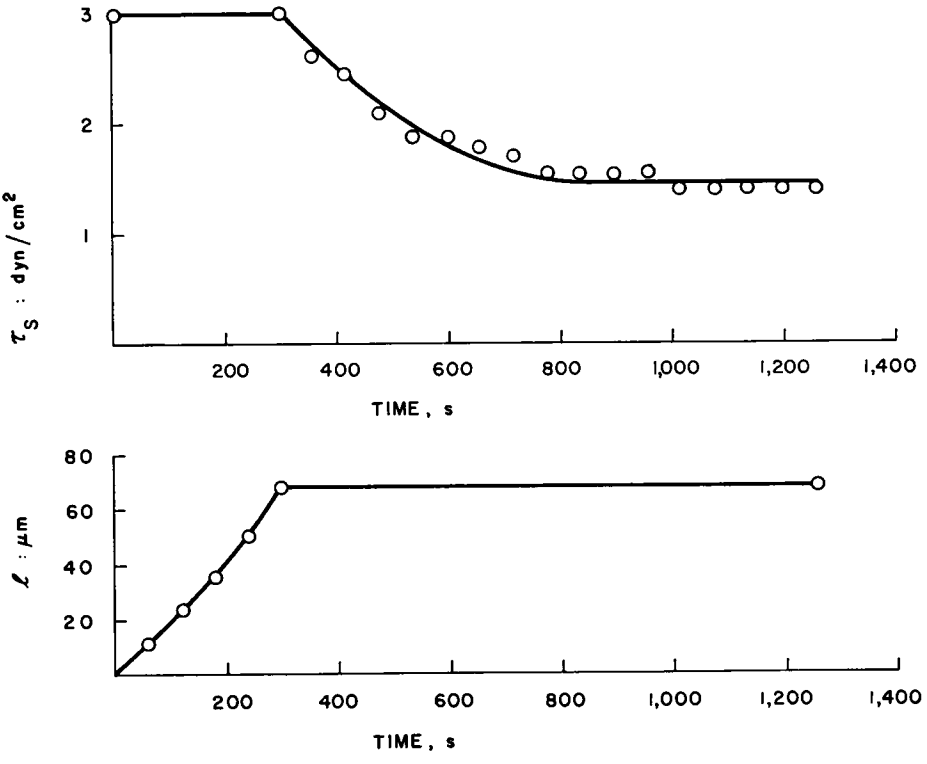

FIGURE 7 Curves showing the increase in tether length l at constant fluid shear stress τ_s and the decrease in τ_s at constant l.

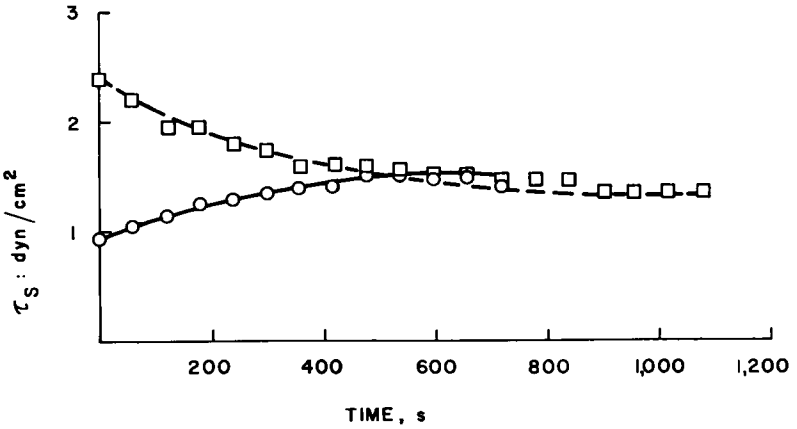

FIGURE 8 The change in fluid shear stress required to keep a given tether at a constant length. The circles represent an experiment in which the initial fluid shear stress was less than the "critical stress for tethering" (approximately 1.5 dyn/cm^2) and therefore the stress had to be increased in order to keep the tether length constant. The squares represent an experiment on the same tether in which the initial stress was greater than the critical stress for tethering.

dyn/cm^2. Overall growth rates varied from 0.03 to 0.20 μm/s for shear stresses between approximately 2.0 and 3.5 dyn/cm^2.

It is felt that the tether growth illustrated in Fig. 7 is caused by the transfer of membrane material from the red cell to the tether. In a sense membrane material flows onto the tether when the critical stress for tethering is exceeded. This fact complicates tether stress-strain measurements (Fig. 5) since an increase in tether length can be caused both by an elastic response of the tether to an increase in stress and by an accumulation of membrane material which in turn undergoes an elastic elongation. For this reason approximately 50% of the stress-strain measurements (Fig. 5) were made at a fluid shear stress between 1.0 and 2.0 dyn/cm^2. In this range the tether length only increases or decreases slightly during the course of a measurement (recall that at 2.0 dyn/cm^2 the overall tether growth rate is on the order of 0.03 μm/s and that length measurements are made at 30, 60, and 90 s).

ANALYSIS

The data for the three types of red cell stretching (whole cell stretching in Fig. 3, stretching of a red-cell evagination in Fig. 4 and tether stretching in Fig. 5) must be analyzed with the use of the free body diagrams shown in Fig. 9 in order to obtain a value from each of these experiments for the elastic modulus of red cell membrane.

Whole Cell and Model Cell Stretching

This type of stretching is illustrated by the photomicrographs shown in Figs. 2 *a* and *d*. A free body diagram of the *top portion* of the cell membrane is shown in Fig. 9 *a*. In the diagram it is assumed that the membrane force $F(x)$, where x is measured from the trailing (free) edge, is constant over the membrane cross section (one-dimensional analysis). Also, the membrane stresses which exist along the curved periphery of the free body diagram are ignored. Finally it is assumed that the shear stress acting over the surface of the membrane is the same as the shear stress at the wall (τ_s) and that the net force acting on the red cell is equal to τ_s times the area of the cell projected onto the surface. This is a good assumption for a flat body. However, Hyman (10) has shown recently that the force on a small *hemispherical* body is equal to four times the product of τ_s with the projected area.

The above assumptions permit the stress in the membrane $\tau(x)$ to be written as

$$\tau(x) = F(x)/tW(x) = \tau_s A(x)/tW(x), \tag{2}$$

where the area $A(x)$ is the integral of the width function $W(x)$ between 0 (the trailing edge) and x, and t is the thickness of the membrane. If it is assumed that the stress is linearly related to the strain $\epsilon(x)$ at any point x in the membrane, then

$$\tau(x) = E\epsilon(x), \tag{3}$$

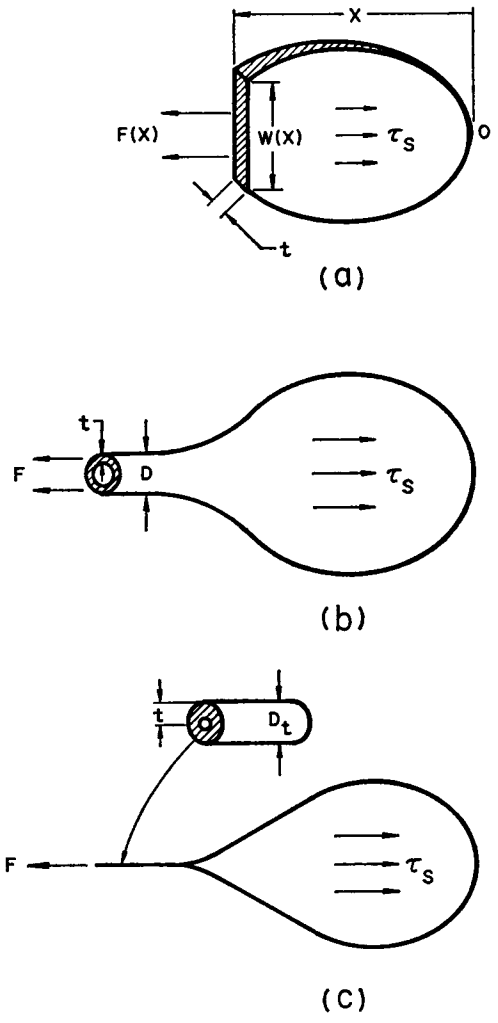

FIGURE 9 Free body diagrams: (*a*) whole cell stretching, (*b*) stretching of an evagination, (*c*) tether stretching.

where E is the modulus of elasticity. The local strain in the membrane can be expressed in terms of the displacement function $u(x)$ as follows:

$$\epsilon(x) = \mathrm{d}u(x)/\mathrm{d}x. \tag{4}$$

Integration of Eq. 4 over the entire length of the membrane yields

$$\int_0^l \epsilon(x)\,\mathrm{d}x = u(x)\,|_0^l = u(l) = l - l_0, \tag{5}$$

since the displacement at $x = 0$ is zero (the origin of the coordinate system has been arbitrarily fixed at the trailing edge of the cell) and the displacement at $x = l$ is equal to the overall change in length of the cell ($l - l_0$). When Eq. 3 is substituted into Eq. 2 and integrated (see Eq. 5), the result is

$$l - l_0 = \frac{\tau_s}{Et} \int_0^l \frac{A(x)}{W(x)}\, dx. \tag{6}$$

The calculation for the modulus E is greatly simplified if it is assumed that the red cell stretches as a rectangular strip. (Fig. 2 *a* shows that this can be a very reasonable assumption.) For a rectangular strip, $W(x) = W_0$ and $A(x) = W_0 \cdot x$. Thus Eq. 6 becomes

$$l - l_0 = (\tau_s/Et)(l^2/2). \tag{7 a}$$

When written in terms of the extension ratio λ ($\lambda = l/l_0$) and rearranged slightly, Eq. 7 *a* becomes

$$E = \tau_s \lambda^2 \Big/ \frac{2t}{l_0} (\lambda - 1). \tag{7 b}$$

For values of λ close to one, the straight line portion of the curve in Fig. 3 is given by

$$\tau_s = 50(\lambda - 1)\,(\text{dyn/cm}^2). \tag{8}$$

The substitution of Eq. 8 into Eq. 7 *b* gives

$$E = \frac{50\lambda^2}{2t/l_0}\,(\text{dyn/cm}^2),$$

$$= 2.5 \times 10^4\ \text{dyn/cm}^2, \tag{9}$$

for $\lambda = 1$, $t = 0.01\ \mu$m and $l_0 = 10\ \mu$m.

The model cell experiments furnished a means of checking the assumptions which led to the theory of cell stretching as given by Eq. 6. This equation is used to calculate a modulus of elasticity for the latex rubber "membrane" and then a portion of the membrane is excised from the model cell and its modulus is measured directly in a simple tension test. In the model cell experiment, a 22% increase in the original length of 4 cm is measured when $\tau_s = 2{,}270$ dyn/cm^2. For these values of $l - l_0$ and τ_s, Eq. 6 gives a value for E of 1.1×10^7 dyn/cm^2 for $t = 100\ \mu$m, $l_0 = 4$ cm and for $A(x)$ and $W(x)$ obtained from the shape shown in Fig. 2 *d*. The "true" (directly measured) value for E was 1.7×10^7 dyn/cm^2.

The above analysis is based on the assumption of infinitesimal deformation even though the cells are undergoing finite deformation (Fig. 2 *a*). Evans[2] has analyzed

[2] Evans, E. A. Manuscript in preparation.

the case of fluid shear deformation of cells with point attachment (6) using finite deformation theory [$\lambda(x) = (1 + 2\epsilon[x])^{1/2}$ rather than $\lambda(x) = 1 + \epsilon(x)$] and a constant thickness, constant surface area membrane model. Evans's theoretical approach predicts the change in shape of point-attached red cells observed by Hochmuth and Mohandas (6) and also predicts the existence of tethers. The shape change and overall elongation of a cell is a function of the single dimensionless parameter $\tau_s l_0/Et$ (see, also, Eq. 7 *b*). However, the point to be made here is that Evans's more general and rigorous theoretical approach still yields a value for the modulus of 10^4 dyn/cm^2 when the experimental data of Hochmuth and Mohandas are used.

Stretching of a Red Cell Evagination

This type of stretching is illustrated by the photomicrograph shown in Fig. 2 b. A free body diagram of the red cell and its evagination is shown in Fig. 9 *b*. Again it is assumed that the net force acting on the red cell is equal to τ_s times the area of the cell projected onto the surface and it is assumed that the area of the evagination is negligible compared with the area of the cell. In this case, the force F in the membrane is

$$F = \tau_s A_c$$

where A_c is the area of the cell. The stress τ in the membrane is

$$\tau = F/\pi Dt = \tau_s A_c/\pi Dt \qquad (10)$$

where D is the diameter of the evagination and t is the membrane thickness. Compare Eqs. 10 and 2. Again the stress and strain are assumed to be linearly related:

$$\tau = E\epsilon. \qquad (11)$$

The substitution of Eq. 11 into Eq. 10 gives

$$E\epsilon = \tau_s A_c/\pi Dt. \qquad (12)$$

The straight line shown in Fig. 4 is given by

$$\tau_s = 3.76\epsilon \text{ (dyn/cm}^2\text{)}. \qquad (13)$$

The substitution of Eq. 13 into Eq. 12 gives

$$E = (3.76A_c/\pi Dt) \text{ (dyn/cm}^2\text{)},$$

$$= 0.86 \times 10^4 \text{ dyn/cm}^2, \qquad (14)$$

for $D = 0.765$ μm (measured with a filar micrometer), $A_c = 55$ μm^2 (typical projected area of a red cell) and $t = 0.01$ μm.

Tether Stretching

This type of stretching is illustrated by the photomicrographs shown in Figs. 2 *c* and 6. A free body diagram for a red cell and its tether is shown in Fig. 9. The analysis of tether stretching proceeds exactly as the one for the stretching of an evagination with D_t, the tether diameter, substituted for D, the evagination diameter.[3] Thus, Eq. 12 is rewritten as

$$E\epsilon = \tau_s A_c/\pi D_t t, \tag{15}$$

where it is still assumed that the membrane thickness t is much smaller than the tether diameter D_t in order that the cross-sectional area of the tether can be written as $\pi D_t t$. The equation for the straight lines shown in Fig. 5 is

$$(\tau_s/\mathrm{E}) = 1.31 \pm 0.38 \ (\mathrm{dyn/cm^2}). \tag{16}$$

The substitution of Eq. 16 into Eq. 15 gives

$$E = \frac{(1.31 \pm 0.38)A_c}{\pi D_t^2 t} \ (\mathrm{dyn/cm^2}),$$

$$= (1.15 \pm 0.33) \times 10^4 \ \mathrm{dyn/cm^2}, \tag{17}$$

for $A_c = 55 \ \mu\mathrm{m}^2$, $t = 0.01 \ \mu\mathrm{m}$, and for $D_t = 0.2 \ \mu\mathrm{m}$ (estimated from optical and scanning electron photomicrographs).

DISCUSSIONS AND CONCLUSIONS

For the three types of red cell-stretching experiments, the value for the modulus of elasticity of red cell membrane was calculated to be on the order of 10^4 dyn/cm^2 (see Eqs. 9, 14, and 17). This value applies to the red cell membrane when it is in a state of uniaxial tension. The relatively complicated technique used to calculate the membrane modulus for whole cell stretching (Eq. 6) appears to be valid since the same technique when applied to a large scale model cell gave a value for the membrane modulus which was within a factor of 0.7 of the true value. Thus it is concluded that the modulus of elasticity for red cell membrane in uniaxial tension is on the order of 10^4 dyn/cm^2 for an assumed constant membrane thickness of 0.01 μm. This value for the modulus of elasticity is three to four orders of magnitude less than the values given by Rand (3) and Katchalsky et al. (4). In both of these experiments the membrane was subjected to a biaxial stress and strain. Thus,

[3] However, the evagination is qualitatively different from a tether. The evagination existed before the stress-strain experiment whereas the tether is formed from a red cell when the shear stress exceeds approximately 1.5 dyn/cm^2. The diameter of the evagination can be measured directly rather than estimated and it is approximately three to seven times the diameter of a tether.

the overall membrane surface area probably increased and this may account for the three to four order-of-magnitude difference. However, Evans[2] has reanalyzed the micropipette experiments of Rand and Burton (1) and has also obtained a value for the modulus of 10^4 dyn/cm^2. He indicates that this lower value is for a "shear" modulus which is three orders of magnitude less than the "bulk" modulus for red cell membrane (3, 4).

The data as shown in Figs. 3–5 also indicate that the membrane behaves as a linearly elastic material when subjected to a uniaxial load. Fig. 5 shows a linear behavior for strains as high as 400%. The data in Fig. 3, however, indicate a nonlinear elastic behavior for values of λ greater than 1.13. This is probably due to the two-dimensional strain which appears in the membrane at higher values of fluid shear stress. The cell membrane is initially loaded uniaxially, but as the shear stress increases the leading edge of the cell (the edge firmly attached to the surface; see Fig. 2 *a*) is not able to contract and consequently the cell membrane is subjected to a biaxial strain. In the experiment involving the tethers, a biaxial strain in the membrane probably does not occur except right after the application of a stress. The sudden application of a stress causes the tether to contract and thus forces hemoglobin from the tether into the red cell. The hemoglobin which does not escape balls up, and the region around these hemoglobin globules are regions of biaxial stress and strain.

It can be claimed that the tether is not representative of whole cell membrane but consists of some component or part of the cell membrane (perhaps the phospholipid). There are several reasons, however, to suppose that the tether consists of whole cell membrane. In the first place the value for the modulus of elasticity of the tether is comparable with that calculated for whole cells and the evagination. Secondly the tether is relatively thick (approximately 0.2 μm) compared with, for example, the double thickness of a lipid film (approximately 0.02 μm). Finally, the tether does seem to communicate with the cell in that the postulated hemoglobin globules are often observed to move up the tether and disappear into the cell.

The fascinating thing about a red cell tether is that it appears to behave both like a fluid and like a solid. When the critical stress for tethering (1.5 dyn/cm^2) is exceeded, a tether forms and grows slowly with time. Tethers with lengths in excess of 200 μm have been recorded on 16 mm film. This means that two points on the red cell membrane which are initially diametrically opposite and 8 μm apart can eventually be over 200 μm apart if one of the points is the point of adhesion of the tether. It would appear that membrane "flows" from the cell to the tether and in the process its structure is rearranged since membrane "points" which are initially close together on the red cell (no two points on a red cell are more than approximately 8.5 μm apart) can be much farther apart on the tether. Yet for a tether of a given length at zero stress, stress-strain experiments can be performed and a modulus of elasticity can be measured. The small value of 10^4 dyn/cm^2 for this modulus indicates a highly elastic material. The fluid and elastic behavior of a tether is not inconsistent

with a model for cell membrane which consists of a fluid-like bimolecular lipid matrix in which protein is embedded (11).

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