MECHANICAL PROPERTIES OF THE RED CELL MEMBRANE

II. Viscoelastic Breakdown of the Membrane

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ABSTRACT A technique is described for measuring the stress required to rupture the red cell membrane. It requires a measure of the pressure and time to suck a cell into a micropipette. If a long tongue is pulled into a micropipette, the membrane spontaneously collapses on itself, as does a cylinder of fluid of length equal to its diameter, and a small refractile globule and a swollen red cell are produced. If the cells are hypotonically swollen enough, they cannot afford enough area for a long tongue to move into the pipette and when stressed for a time, hemolyse and disappear into the pipette. Calculation of membrane tension shows that the membrane can withstand a wide variety of tensions, up to a maximum of 20 dynes/cm for short periods of time, but even at much lower stresses the membrane eventually either ruptures, and the cell hemolyses, or relaxes; there does not appear to be a yield stress. This observation and the kinetics of the cell breakdown lead to a viscoelastic model of the cell membrane. The membrane substance has a Young's modulus of approximately 10^6 to 10^8 dynes/cm^2 and a viscosity of 10^7 to 10^{10} poises. This confirms and extends the viscoelastic model proposed by Katchalsky *et al.* (3).

INTRODUCTION

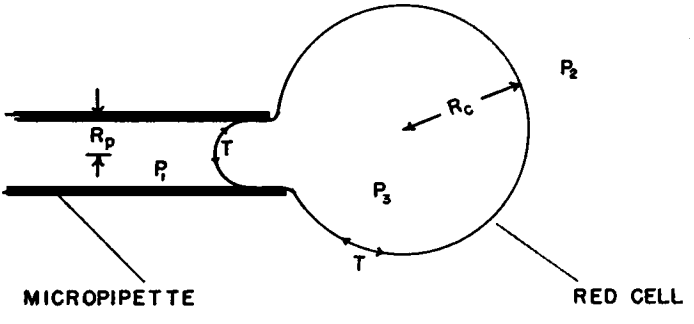
Previously we reported results on measurements made on the "stiffness" of the red cell membrane (1). Following Mitchison and Swann (11), we use the term stiffness as meaning resistance to deformation resulting from both rigidity and tension of the membrane; both resistances being present in the membrane of the red cell (1). Stiffness was measured on hypertonically crenated cells, normal biconcave discs, and cells that were hypotonically swollen, but not sphered. Stiffness never exceeded the low value of 0.018 dynes/cm. It has been noted, however, that whenever the area of the cell membrane was made to increase, it became extremely "stiff." This large increase in stiffness can be observed in hypotonically sphered, but not hemolyzed cells (also observed by Ponder, 2), and also can be observed when a cell is sucked into a micropipette until the part of the cell remaining outside the pipette is the portion of a sphere (see Fig. 1 in Reference 1). In both these cases the area of

the membrane must increase before further deformation can occur. An attempt to measure this very large increase in stiffness over that of the undeformed cell led to this report of the viscoelastic breakdown of the cell resulting in hemolysis.

Katchalsky *et al.* (3) have considered a mechanical rheological model of the hemolyzing red cell membrane derived from experiments on gradual osmotic hemolysis *versus* drastic or fast osmotic hemolysis. Their model of the cell membrane that best describes the kinetics of the membrane breakdown consists of a Kelvin body, *i.e.* a spring and dash-pot, representing elastic and viscous components respectively, fixed in a parallel arrangement. Assuming that the model breaks, *i.e.* the membrane stretches and the cell hemolyzes, at a critical membrane tension, the authors derived values for the elastic and viscous moduli for the membrane substance. Our experiments indicate that hemolysis occurs at a variety of membrane tensions and suggest that hemolysis occurs at a critical strain or stretching of the cell membrane rather than at a critical tension. The results also indicate that the model must include, in series, a dashpot or viscous component since the membrane appears able to flow and hence hemolysis occurs under low stresses.

METHOD

The technique used was modified from that previously described in detail (1) and used to measure the resistance to deformation or stiffness of the membranes of red cells. Briefly, it requires a measure of the pressure and time required to draw a cell into a micropipette (see Fig. 1). Red cells, obtained from a finger prick, were suspended in a 0.65 per cent



$$P_3 - P_1 = \frac{2T}{R_p} \qquad P_3 - P_2 = \frac{2T}{R_c}$$

$$\therefore \; P_2 - P_1 = 2T\left(\frac{1}{R_p} - \frac{1}{R_c}\right) \qquad (1)$$

FIGURE 1 Schematic diagram of a red cell in a hanging drop that has been drawn into a micropipette of radius R_p until the outer part is the portion of a sphere of radius R_c. P_1, P_2, and P_3 are the pressures inside the pipette, inside the hanging drop and inside the cell respectively.

buffered (pH = 6.2) saline solution to swell the cells hypotonically to the desired degree but not to sphere them. The hematocrit was made very low by the saline dilution so that individual cells could be studied in a hanging drop. After approximately 15 minutes, a small drop of the suspension was placed on a coverslip and immediately covered with paraffin oil to prevent evaporation. In approximately 5 minutes the coverslip was inverted and placed on a chamber to form a hanging drop (Fig. 2). With the aid of a

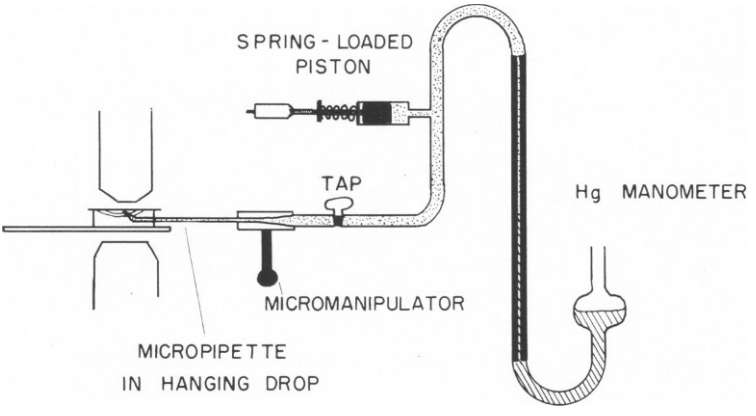


FIGURE 2 Apparatus used to suck the cells into a micropipette. Negative pressure is produced by the spring-loaded piston which is driven by hand with a micrometer. The pipette is positioned in the hanging drop with the aid of a micromanipulator.

micromanipulator a micropipette could then be placed against those cells which were found hanging from the underside of the coverslip. Under low pressure (0.5 mm Hg) the cell could be sucked into the micropipette to plug it, and the part remaining outside the pipette became the portion of a sphere. The tap (Fig. 2) was closed and the pressure P_1 (Fig. 1), measured with a mercury manometer, was reduced to a desired level with the aid of a spring-loaded piston and cylinder that was hand-driven with a micrometer movement. The tap was then opened to pull the cell into the pipette under pressure $P = (P_2 - P_1)$. The cell did not immediately move further into the pipette, but in time t, measured from the time the tap was opened, the cell suddenly disappeared into the pipette. Occasionally P was then made positive and the cell ghost flowed easily out of the pipette under low pressure P, *i.e.* $(P_2 - P_1) \approx -1$ cm Hg, and appeared as a flaccid deformed biconcave ghost. P and t were determined over a wide range of t (up to 200 seconds) varying P over this whole range between 5 to 20 times giving an average t for 5 to 20 cells for each pressure. At all pressures a check was made periodically to be sure the cell plugged the pipette and no flow resulted. This could be done by observing the movement of other cells or debris in the region of the plugged pipette. Determination of the zero pressure level could be accurately made ($\pm$ 0.1 mm Hg) by observing the motion of a cell ghost inside an unplugged pipette. This whole procedure was repeated with five different pipettes. With a sixth pipette, at two different pressures only, a large number of determinations of t were made to investigate the nature of its scatter.

RESULTS

(*a*) *Behaviour of the Cells.* It was critical to what degree the cells were swelled before determinations were made on hemolyzing the cells by sucking them into the pipette. First, if the cells were not swelled enough, the area to volume ratio of the cell was high enough so that the cell could afford a long narrow cylindrical tongue to move into the pipette before the outer portion became spherical in shape. If the cylinder was long enough, the membrane spontaneously collapsed on itself in the middle of the cylinder and sealed (Fig. 3). A highly refractile (hemoglobin-containing?) globule passed down the pipette and the remaining portion was a normally shaped, but a more swollen, unhemolyzed cell. This could be repeated as

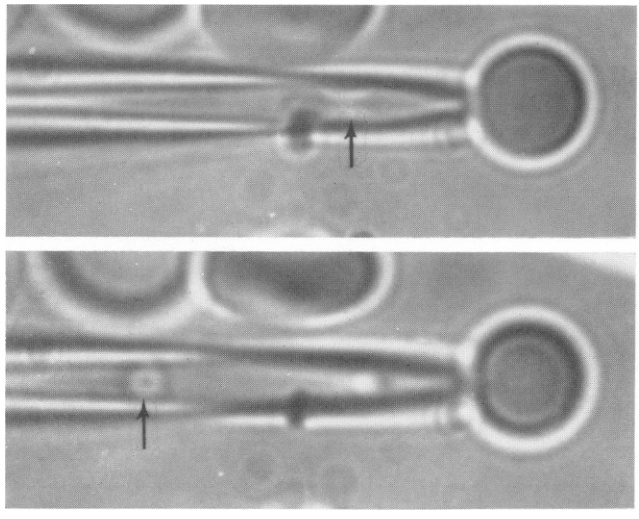


FIGURE 3 Illustrating the spontaneous collapse of the cell membrane on itself when pulled into a long cylinder. Arrows point to the beginning of collapse at the neck of the cylinder and to the refractile globule which finally forms separate from the rest of the cell. The outer spherical portion of the cell is about 3 μ in diameter.

many as four times on the same cell providing the area to volume ratio was high enough to allow a long tongue to move into the particular pipette. Under these circumstances and when the treatment was not so drastic as to increase the cell area to the point where the cell crenated after release (1), it appeared that the shape of the remaining cell was a symmetrical swollen disc whether the globules were removed from the "dimple" region or rim region of the cell. (Copley (4) referred to the observation of this pinching-off phenomenon, *in vivo,* in minute openings in the living capillary wall, one particle being carried off in the circulation and the other remaining outside the cell wall.) It appears that the spontaneous collapse and sealing off of the membrane is similar to the spontaneous collapse and formation of drops

of a fluid cylinder when its length becomes equal to its circumference. It is an indication that the laws of surface tension or membrane elastic tension (6, 7) apply to the cell in these circumstances and at least the tongue of the cell does behave like a fluid-filled membrane.

Secondly, if the cells were swelled too much, frequently they did not completely plug the pipette mouth and flow of saline into the pipette could be detected. Most of the cells had passed from the more flaccid swollen ellipsoid shape to the perfectly spherical but rigid and undeformable condition. If, however, this suspension was observed after some time (2 hours), no cells remained in the highly rigid state. Of the field of swollen spherical cells, two populations of cells remained, hemolyzed flaccid ghosts and unhemolyzed non-spherical flaccid red cells. No rigid spheres were present. This behaviour was observed many times and indicates that the sphered cells do not maintain the very "stiff" membrane for long periods of time but either hemolyze or revert to the flaccid swollen condition. Although it is not known what volume changes these cells undergo, this relaxation of stiffness could support the idea that the membrane is viscoelastic.

Thirdly, if no perfect spheres were present and the cells were swelled adequately, the cells plugged the pipette and no further change could be detected until they suddenly disappeared into the pipette. No adhesion between the cell and pipette could be demonstrated as it could in the case of unswelled cells (1) and in the case when a long tongue could move into the pipette. Fig. 4 gives two histograms illustrating the degree of scatter of times for two pressures or tensions where an exceptionally large number of determinations of t were made. Note the difference in scale of the abscissa. The difference between the mean times at the two different stresses is significantly different from zero with $P \ll 0.1$ per cent. The scatter is considered to be primarily the result first of variation in the population of cells in normal blood and secondly of variation in the degree of swelling of different cells, which determines the size of the spherical portion of the cell (Fig. 1). (See Calculation of Membrane Tension; *b* following.)

In a few isolated trials, under very low stresses ($T \approx 2$ dynes/cm) cells took as long as 2 hours to enter the pipette.

(*b*) *Calculation of Membrane Tension.* Although rigidity, or resistance to bending, of the membrane is as significant as tension in the membrane for the normally shaped cells (1), the law of Laplace, or rather the equilibrium equation for thin membranes (6, 7), was shown to apply. Mitchison and Swann (11) also found this to be the case with their model experiments with rubber balloons. For highly stressed cells the presence of very accurately spherical cells or portions of the cell indicated that tension is the major stress and the same equation was used to calculate this tension.

Consider the geometry of the system in Fig. 1. R_p and R_c are the radii of curvature of the hemispherical cap of the portion of the cell inside the pipette and of the

spherical portion of the cell outside the pipette respectively, P_1, P_2, and P_3 are the hydrostatic pressures inside the pipette, inside the hanging drop, and inside the cell respectively. $P_2 - P_1$ is the measured pressure P described in the method. T is the tension in the membrane assumed to be constant over the whole membrane.

$$\text{Then,} \quad P_3 - P_2 = \frac{2T}{R_c} \quad \text{and} \quad P_3 - P_1 = \frac{2T}{R_p}$$
$$\therefore \quad P_2 - P_1 = 2T\left(\frac{1}{R_p} - \frac{1}{R_c}\right) \tag{1}$$

T can be calculated from this equation. $(P_2 - P_1)$ can be measured very accurately ($\pm$0.2 mm Hg). Measurement of R_p and R_c are made with a calibrated eyepiece micrometer with an error of approximately 10 per cent (1). However, for each individual cell the value of R_c depends on the absolute cell volume and on degree of swelling or shape of the cell; the latter determines the area to volume ratio, and hence how long the tongue will be, and both determine how large the spherical portion will be. These variations give rise to variations in T calculated according to equation (1). These and differences in the population of cell membranes are felt to be the major reason for the large scatter observed in Fig. 4.

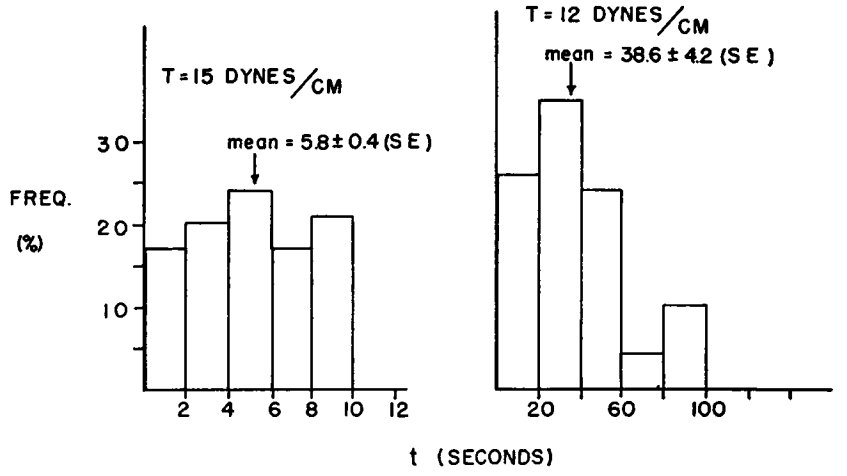


FIGURE 4 Frequency distributions of time for hemolysis using the same pipette at two different pressures or tensions. Note the difference in scales of the abscissa.

Five separate plots of $1/T$ *versus* average t are given in Fig. 5 for the five different pipettes. Note the duplication of the ordinate scale to separate the plots. The range of pipette size is restricted by the behaviour of the cells as described in preceding (*a*). The difference in curves, particularly their relative vertical displacement, is not related to the radius of the pipette, as shown in Fig. 5, but may well be the

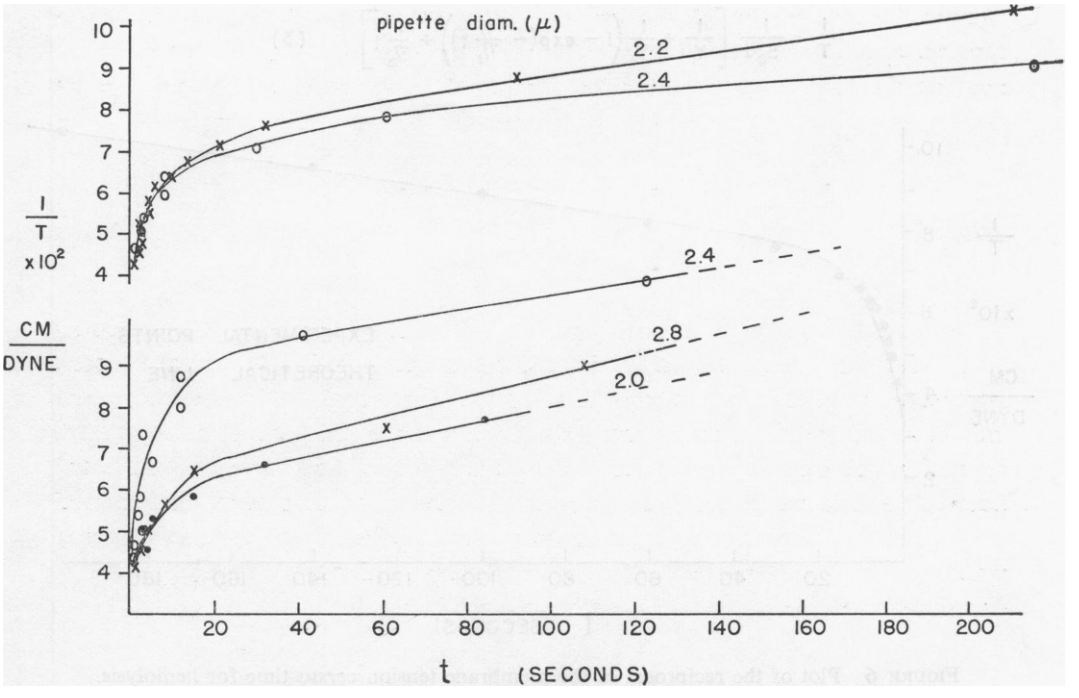


FIGURE 5 Plot of the reciprocal of the membrane tension *versus* the average time taken for hemolysis for the five different pipettes used. Note the duplication of the ordinate to separate the plots.

result of the error in measurement of the pipette radius and of the spherical portion of the cell as indicated above. The important thing to note is that the shape of the curves is the same. The reason for plotting the variables in this fashion will become clear when the membrane model is considered. Mean values of $1/T$ for various t's were obtained graphically from these five curves and the resultant mean curve plotted as the experimental points of Fig. 6.

Because the cell in the pipette does not immediately appear to change when the high pressures are applied, and then hemolyzes and disappears suddenly into the pipette, the cell does not detectably increase in volume over the time period t. It is felt that hemolysis is caused by the applied mechanical stresses. The relation between the tension and time shown in Fig. 6 suggests that the response of the membrane to a step increase of tension is similar to that of a specific viscoelastic body or model that has been applied to a variety of molecular fabrics and polymer solutions (8).

(*c*) *Viscoelastic Model of the Cell Membrane.* Although molecular interpretations of stress-strain relationships in polymer solutions and other viscoelastic media have been made, (5, 8, 9), and in some cases elastic and viscous elements of

$$\frac{1}{T} = \frac{1}{S_c d}\left[\frac{1}{Y_1} + \frac{1}{Y_2}\left(1 - \exp\left(-\frac{Y_1}{\eta_1}t\right)\right) + \frac{1}{\eta_2}t\right] \qquad (3)$$

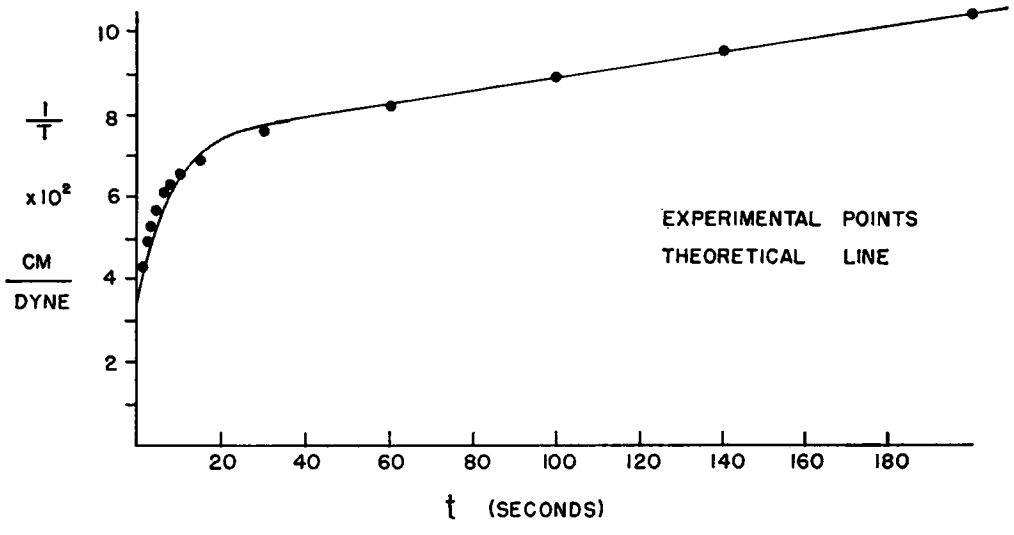


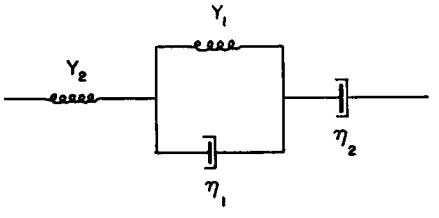
FIGURE 6 Plot of the reciprocal of the membrane tension *versus* time for hemolysis. The experimental points were obtained from the means of Fig. 5. The theoretical line was obtained from the postulated viscoelastic model of Fig. 7 using the elastic and viscous parameters given in the text.

a model confidently identified with some molecular processes, implications concerning the molecular mechanism of hemolysis cannot easily be derived from the phenomenological observations as given here. Because of the lack of information that can be obtained regarding the strain or stretching of the membrane, the model given below will then be considered only as a description of the membrane breakdown behaviour. A viscoelastic model which best fits the curve of Fig. 6 is similar to one used by Alfrey and Gurnee (8) in discussing the dynamics of the viscoelastic behaviour of a polysulfide rubber.

The mechanical model is shown in Fig. 7. It consists of a purely elastic element, conventionally represented by a spring; a purely viscous component, conventionally represented by a dash-pot; and a parallel arrangement of each, all in series. We desire the strain or relative degree of stretch, *i.e.* response of this model, to an applied step function of stress.

Classically, for the individual components the response of the elastic element to a sudden constant stretching force F, measured per unit cross-sectional area of the element, is an immediate increase in length ΔL from an initial length L_0 to its final value, and $F = YS$ where F is in dynes/cm², S is the strain or relative change in

$$S = F\left[\frac{1}{Y_2} + \frac{1}{Y_1}\left(1 - \exp\left(-\frac{Y_1}{\eta_1}t\right)\right) + \frac{1}{\eta_2}t\right] \quad (2)$$



VISCOELASTIC MODEL OF MEMBRANE

FIGURE 7 Mechanical model of the cell membrane used to describe the kinetics of the membrane breakdown and to obtain the theoretical line of Fig. 6.

length of the element, $\Delta L/L_0$, and Y is Young's modulus. The response of a single dash-pot to a sudden force or stress is a steady increase in length with time, and $dS/dt = F/\eta$ where t is time in seconds and η is the viscosity in poises. Thus, $Y/\eta = dS/dt/S$ and has the dimensions of the reciprocal of time (sec.$^{-1}$).

The response of the combination of these elements, shown in Fig. 7, to a step function applied stress is well known and is most easily solved using the Laplace transformation. The result, using the mechanical parameters of Fig. 7, is shown in equation (2).

$$S = F\left[\frac{1}{Y_2} + \frac{1}{Y_1}\left(1 - \exp\left(-\frac{Y_1}{\eta_1}t\right)\right) + \frac{1}{\eta_2}t\right] \quad (2)$$

This gives us the relation between the stress F applied to the system and the strain, S, the system undergoes in response to this stress as a function of time t. The exact relation of course depends on the elastic (Y_1, Y_2) and viscous (η_1, η_2) parameters. If the model is subjected to a specific stress F_1, immediately the series elastic component stretches to its final value ($S = F_1/Y_2$), the parallel arrangement increases in length exponentially, $S = F_1/Y_1\ (1 - \exp\ (-Y_1/\eta_1 \cdot t))$ with time constant Y_1/η_1, while the series viscous component increases steadily in length with time, ($S = F_1/\eta_2 \cdot t$). The combined response given by equation (2) is shown graphically in Fig. 8 for different applied stresses.

It must be remembered, however, that in the case of the red cell the only response that can be measured is the time taken for hemolysis. In fact, no change in the cell can be seen with time after the stress is applied except finally the disappearance of the cell resulting in its hemolysis. This means that the strain S in equation (2) and Fig. 8 cannot be observed, except that it produces the molecular changes or strains

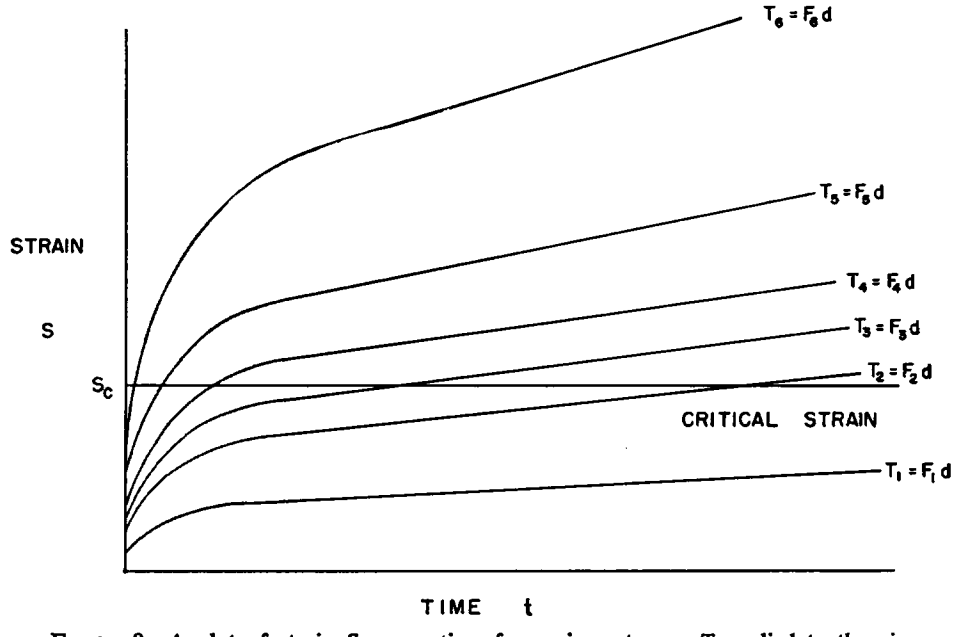


FIGURE 8 A plot of strain S *versus* time for various stresses T applied to the viscoelastic model of Fig. 7, using arbitrary viscous and elastic parameters. This is the family of curves represented by equation (2). The relation between tension and time given by the intersection of the horizonal line S_c with the family of curves is given by equation (3) and plotted in Fig. 6.

that result in breakdown of the membrane. If we make the assumption that hemolysis occurs at a given strain, *i.e.* the cell hemolyzes at a critical relative increase in area or volume (10), the membrane breaks down, hemoglobin is released, and the cell moves into the pipette at a critical strain or stretching of the membrane, S_c, say, then F and t become the only two variables in equation (2). In addition, the thickness of the membrane, d, must be estimated in order to relate the experimentally determined tensions T, (Fig. 6 and equation 1) to the forces F in the membrane. Then $F = T/d$ and $S = S_c$ and equation (2) becomes

$$\frac{1}{T} = \frac{1}{S_c d}\left[\frac{1}{Y_2} + \frac{1}{Y_1}\left(1 - \exp - \frac{Y_1}{\eta_1} t\right) + \frac{t}{\eta_2}\right] \tag{3}$$

This is the function that describes the relation between the stresses and times represented by the intersection of the horizontal line S_c in Fig. 8 with the family of curves shown. This function can be fitted to the empirical data. When estimates from the experimental points of Fig. 6 are made of: (a) the intercept on the vertical axis ($1/Y_2 S_c d$), (b) the slope of the linear portion of the curve ($1/\eta_2 S_c d$), (c) the time constant of the exponential rise (Y_1/η_1), and (d) the final value of the exponential rise ($1/Y_1 S_c d$), the theoretical curve in Fig. 6 is obtained and a good fit made to

the experimental data. No set of parameters could be found that gave a good fit on the assumption that the exponential portion of the curve passes through the origin. On this basis it is felt that the experimental curves do in fact intercept the axis and consequently a series elastic component must be included in the model. The non-zero slope of the linear portion of the curve implies that a series viscous component must be included. The parameters which give the theoretical curve of Fig. 6 are:

$$1/Y_2S_cd = 3.5 \times 10^{-2} \text{ cm/dyne} \qquad 1/Y_1S_cd = 3.9 \times 10^{-2} \text{ cm/dyne}$$

$$Y_1/\eta_1 = 8 \text{ sec.}^{-1} \qquad 1/\eta_2S_cd = 1.5 \times 10^{-4} \text{ cm/dyne sec.}$$

These four equations involve six unknowns (Y_1, Y_2, η_1, η_2, S_c, d)

(*d*) *Estimation of the Elastic and Viscous Components of the Cell Membrane.* In order to make an estimate of the four mechanical parameters of the membrane model, estimates of membrane thickness, d, and of the critical strain, S_c, must be made.

We have an estimate of S_c. Previously we made measurements of the area changes involved in hemolyzing single red cells (10). Also, our measurements on cell membrane tension suggest that no significant stresses operate in the membrane until the area is made to increase (1); *i.e.*, until the cell becomes spherical. On the basis then of the mean initial and final areas of the hemolyzing spherical cells, the mean area changed from 146 μ^2 to 171 μ^2 (an increase of 25 $\pm$ 10 μ^2 in area). This corresponds to a change in radius from 3.41 $\times$ 10^{-4} cm to 3.69 $\times$ 10^{-4} cm (an increase of 0.28 $\times$ 10^{-4} cm). However, difficulty arises in defining strain of the membrane model in terms of the strain of the membrane. For example, do the two ends of the model find themselves attached to two points in the plane of the membrane (*i.e.* a tangential arrangement) or one attached to the inside and the other to the outside of the membrane? The question cannot be answered (no molecular structures are represented by the springs and dash-pots) and this interpretation is too naive to be meaningful. However, two estimates of strain most probably define its extreme limits; first, the square root of the relative increase in membrane area ($(25/146)^{\frac{1}{2}} = 0.42$) and secondly, the relative increase in cell radius 0.28/3.41 = 0.08). This latter is the one used by Katchalsky *et al.* (3) based on cell volumes calculated from osmotic data.

Estimates of membrane thickness range from 100 A to 1000 A (2). Using these limits of S_c and of membrane thickness and assuming that in this experiment cells hemolyze after this degree of strain, the absolute values of the elastic and viscous parameters were calculated and the extreme limits are shown below.

Membrane thickness	d = 100 *A* to 1000 *A*
Critical strain	S_c = 0.08 to 0.42
Young's modulus	$Y_1 \approx Y_2 = 7.3 \times 10^6$ to 3.0×10^8 dynes/cm^2

Viscosity $\eta_1 = 5.8 \times 10^7$ to 2.4×10^9 poises

$\eta_2 = 1.6 \times 10^9$ to 9×10^{10} poises

The range in these values could be reduced, of course, if a narrower range in thickness of the cell membrane could be held responsible for the mechanical properties and an observation of the actual strain of the membrane could be made.

These values compare favourably with those obtained by Katchalsky *et al.* (3) who used a completely different technique.

$$Y = 2.4 \times 10^7 \text{ dynes/cm}^2$$

$$\eta = 2.7 \text{ to } 27 \times 10^7 \text{ poises.}$$

From these measurements, *and for comparison purposes only,* the membrane substance has a viscosity between that of wax and pitch, at 10°C, and has a Young's modulus close to that of rubber (4×10^7) and between that of elastin (3×10^6) and collagen (10^9).

DISCUSSION

The empirical curve representing mechanical strain and breakdown of the membrane fits well the postulated viscoelastic model. It must be emphasised that a large gap exists between a molecular interpretation of the breakdown of the cell membrane and the empirical data. The experimental data at this stage permits the model to be used only as a means of describing the data.

The fundamental differences of the present model with that proposed by Katchalsky *et al.* (3), which was made up of only the parallel arrangement in our model, are as follows:

1. The basic hypothesis in our model is that hemolysis occurs at a critical strain, rather than stress, of the cell membrane. That this is so is supported by the fact that the membrane can withstand a wide variety of tensions (as calculated by equation 1 and indicated in Fig. 6) for limited periods of time without rupture.

2. The significant slope of the linear portion of the emperical curve suggests that a series viscous component exists and that the membrane can flow. Also, a few isolated trials showed that the cell may take as long as 2 hours to hemolyze under a low stress. Any flow must exist only on the molecular level, since no strain can actually be observed. Permanent flow on the molecular level does not necessarily involve destruction of the shaping forces of the cell as these may be quite independent and much longer range forces. Hence the hemolysed cells can still revert to the flaccid biconcave ghost as observed.

The model of Katchalsky *et al.* was derived from data which clearly indicated that osmotic hemolysis carried out quickly occured at a higher salt concentration than osmotic hemolysis carried out in a solution which slowly decreased in tonicity.

This indicated that hemolysis depended on the rate at which water entered the cell. These authors assumed that in the case of slow water entry the cell increases in volume more than on fast water entry, and for this latter case the cell bursts before much inflation occurs. Equally plausible is the assumption that the cell inflates to the same degree or hemolyzes at the same degree of strain, for fast or slow swelling; the series viscous component being primarily strained in slow swelling, the series elastic component being primarily strained on fast hemolysis. Only on this basis can the present model account for the results of both sets of data.

Other models may well be suggested. For example, we have made no suggestions of how the tension to which the membrane is subjected affects water permeability. We have assumed purely mechanical breakdown of the membrane. However, since no molecular interpretation is being made at this stage and since the models are used only to describe results of experimental data, nothing can yet be gained by preferring one of the models suggested to the other. Each describes the breakdown of the cell membrane by completely unrelated techniques and indicates that the membrane behaves as a viscoelastic solid. Values for the elastic and viscous moduli of the red cell membrane compare favourably.

CONCLUSIONS

For the purpose of our investigation of the mechanical behaviour of the cell membrane our results can be summarized. We have identified two changes which take place in the membrane as the cell swells osmotically from a crenated sphere through the normal shape to a perfect sphere. The first, during a change in shape from a crenated sphere to a normally shaped or a swollen cell, was described in a previous communication (1). This shape change is accompanied by a change in stiffness from pure rigidity of the membrane equivalent to 0.007 dynes/cm with zero internal pressure to a stiffness involving both rigidity and tension in the membrane equivalent to 0.018 dynes/cm with an intracellular pressure of approximately 2 mm H_2O. These latter values exist for the normally shaped cell whether determined on the rim or biconcavity of the cell and persist right until the cell is swollen just short of the spherical shape. From this, the reason for the normal biconcave shape remains obscure. It appears that in this first change some reorganization occurs in the membrane and that when a small pressure develops inside the cell the stiffness increases as if some "slack" has been taken out of some structural member of the membrane. This degree of stiffness then persists as the cell swells until the membrane is made to increase in area osmotically or mechanically as described herein. At this point a second change or reorganization occurs and the cell suddenly pops into the perfectly spherical shape (1, 2) and becomes extremely stiff. The membrane then exhibits tensions as high as about 20 dynes/cm and viscoelastic breakdown of the membrane occurs if the stress is continued. There does not appear to be a yield stress. The high values of Young's modulus, 10^6 to 10^8 dynes/cm^2, and of viscosity,

10^7 to 10^{10} poises, suggest that the membrane substance must be thought of as a tough viscoelastic solid.

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