TEMPERATURE DEPENDENCE OF THE YIELD SHEAR RESULTANT AND THE PLASTIC VISCOSITY COEFFICIENT OF ERYTHROCYTE MEMBRANE

Implications About Molecular Events During Membrane Failure

R. E. WAUGH

Department of Radiation Biology and Biophysics, University of Rochester, School of Medicine and Dentistry, Rochester, New York 14642

ABSTRACT Structural failure of the erythrocyte membrane in shear deformation occurs when the maximum shear resultant (force/length) exceeds a critical value, the yield shear resultant. When the yield shear resultant is exceeded, the membrane flows with a rate of deformation characterized by the plastic viscosity coefficient. The temperature dependence of the yield shear resultant and the plastic viscosity coefficient have been measured over the temperature range 10–40°C. Over this range the yield shear resultant does not change significantly (± 15%), but the plastic viscosity coefficient changes exponentially from a value of 1.3×10^{-2} surface poise (dyn s/cm) at 10°C to a value of 6.2×10^{-4} surface poise (SP) at 40°C. The different temperature dependence of these two parameters is not surprising, inasmuch as they characterize different molecular events. The yield shear resultant depends on the number and strength of intermolecular connections within the membrane skeleton, whereas the plastic viscosity depends on the frictional interactions between molecular segments as they move past one another in the flowing surface. From the temperature dependence of the plastic viscosity, a temperature-viscosity coefficient, E, can be calculated: η_p = constant × $\exp(-E/RT)$. This quantity (E) is related to the probability that a molecular segment can "jump" to its next location in the flowing network. The temperature-viscosity coefficient for erythrocyte membrane above the elastic limit is calculated to be 18 kcal/mol, which is similar to coefficients for other polymeric materials.

INTRODUCTION

The erythrocyte membrane exhibits both solid- and liquid-like behavior. As long as the maximum shear resultant[1] in the surface remains below some critical value, the membrane behaves elastically, but if the critical value is exceeded, the membrane flows like a liquid. This behavior led Evans and Hochmuth (1976) to model the membrane as a two-dimensional Bingham material. They defined the yield shear resultant, $\hat{T}_s$, as the largest shear resultant which the membrane can support elastically. When the maximum shear resultant in the surface is less than the yield shear resultant, the extension of the surface at a material point, λ, is related to the maximum shear resultant at that point, T_s, by the surface elastic shear modulus, μ:

$$T_s = (\mu/2)(\lambda^2 - \lambda^{-2}) \quad T_s \leq \hat{T}_s. \tag{1}$$

When T_s is $> \hat{T}_s$, the membrane flows. The rate of deformation, V_s, is related to the excess shear resultant, $T_s - \hat{T}_s$, by the plastic viscosity coefficient, η_p:

$$T_s - \hat{T}_s = 2\eta_p V_s \qquad T_s > \hat{T}_s. \tag{2}$$

This equation defines the plastic viscosity coefficient, η_p. Hochmuth et al. (1976) obtained a measure of the yield shear resultant and plastic viscosity for normal cells at room temperature. The present report presents data on the temperature dependence of the yield shear resultant and plastic viscosity of normal erythrocyte membrane.

Measurement of the temperature dependence of material properties can provide insight into the molecular events that occur in deformation. In a previous study, the temperature dependence of the elastic shear modulus was used to resolve the free energy change with deformation into its entropic and enthalpic contributions (Waugh and Evans, 1979). The yield shear resultant depends on the number and strength of the intermolecular connections within the membrane skeleton. Measurement of the temperature dependence of the yield shear resultant provides information about changes in the membrane skeleton that might occur with changing temperature. Measurement of the temperature dependence of the plastic viscosity allows the determination of the temperature-viscosity coefficient,

[1]Resultant is used in the mechanical sense of a force per unit length.

which gives a measure of the extent of the molecular rearrangements which occur in the flowing membrane.

METHODS

The method for measuring the yield shear resultant and plastic viscosity coefficient is the flow channel technique developed by Hochmuth and co-workers (1973) and subsequently analyzed by Evans and Hochmuth (1976). A thin channel was formed between two glass surfaces separated by a parafilm gasket. The channel was held together by a brass frame that contained ports to allow fluid to enter and leave the chamber. The assembly was placed on the stage of an inverted microscope. The bottom glass surface consisted of a cover glass. In the original experiments, the upper surface consisted of a glass slide. In these experiments, the glass slide was replaced by a chamber through which temperature controlled water could flow. The surfaces of this chamber were glass, so that the cells could be illuminated from above. Flow was induced in the channel by raising and lowering two reservoirs. The pressure drop in the channel was monitored with a differential pressure transducer with an accuracy of ±0.5%. The temperature was monitored by two small thermocouples placed at the entrance and exit of the channel. The fluid in the reservoirs was heated or cooled, depending on the experimental temperature, but the fluid tended to come to room temperature as it flowed from the reservoir to the chamber. Because of this, the upstream temperature shifted slightly toward room temperature at high flow rates. The magnitude of the shift with upstream temperature was not more than 1.0°C, and the downstream temperature did not change no matter how large the flow rate in the channel, so the temperature of the cells probably did not change by more than 0.5°C.

Cells were drawn in heparin and separated from the plasma by centrifugation. The plasma was removed and saved and the buffy coat was discarded. Cells were washed once in isotonic phosphate-buffered saline (130 mM NaCl, 6.2 mM KH_2PO_4, 25 mM $Na_2H\ PO_4$) plus 10.0 μM azide (pH 7.3). Cells were resuspended in same buffer containing 0.1 g/100 ml human serum albumin (HSA) to a hematocrit of 1.0–1.5%. A suspension of 8.0% plasma in phosphate buffer was also prepared. Air bubbles were removed from the chamber by flushing with HSA-containing buffer. Then the 8.0% plasma solution was infused for ~2 min to coat the glass surfaces. The plasma solution was flushed out with buffer and the cell suspension was infused into the chamber. Cells were allowed to settle and stick undisturbed for 25–30 min, at which time unattached cells were flushed out of the chamber. Once the chamber was cleared, flow was reversed and then oscillated gently to locate single point attached cells. These cells were positioned in the field of view of a TV camera attached to the microscope, and measurements were begun. The flow rate was increased to some fixed value and held constant for ~1 min, after which the flow was reversed to check for multiple attachments, then increased again to some new fixed value. This process was repeated until the cells became detached from the glass and were lost from view. Experiments were recorded on video tape for subsequent analysis. The rate of tether growth was measured as a function of the pressure drop in the chamber, as indicated by the pressure transducer. The output of the transducer along with the temperature, time and frame number were displayed on the television monitor and recorded with the image of the cells.

CALCULATIONS

Evans and Hochmuth (1976) have analyzed the tether growth process to obtain intrinsic membrane material properties from experimental parameters. The quantities that are measured in the experiment are the pressure drop across the channel, ΔP, and the rate of tether growth, dL/dt. The fluid shear stress on the surface of the cell is approximately equal to the shear stress at the wall of the channel, τ_w, which is related to the pressure drop by:

$$\tau_w = (\Delta P h)/2l, \tag{3}$$

where h is the thickness of the channel and l is its length. The force on the cell is approximated by the product of the shear stress at the wall and the projected area of the cell A_p:

$$F \simeq \tau_w \cdot A_p. \tag{4}$$

The projected area is taken as the area of a disk 8.0 μm in diameter (50 μm^2). Thus, the force on the cell can be calculated from the chamber dimensions and the measured pressure drop.

Evans and Hochmuth (1976) used the constitutive equations (Eqs. 1 and 2) and balance of forces to solve for a relationship between the ratio of the force on the cell to the critical force, F/F_c, and a dimensionless tether growth parameter, G_t:

$$G_t = \frac{8\pi\eta_p}{F_c}\frac{dL}{dt}. \tag{5}$$

The critical force is the maximum force that the tether can support at constant length. For given values of the critical force and plastic viscosity a unique relationship between force and tether growth rate is specified. This relationship is shown in Fig. 1. If the value of the critical force is known, the plastic viscosity can be calculated directly from this relationship for each data pair, (F, dL/dt). When more than one data pair is available for a given cell, a single parameter least squares can be used to obtain the best value for the viscosity. However, experimental determination of the critical force is prone to error because of differences in the area of the attachment site and the elastic nature of a preformed tether. Therefore, an alternative method of calculation was developed, (Waugh and La Celle, 1980) which uses two-parameter least squares to calculate both the critical force and the plastic viscosity. Unfortunately, there was not always enough resolution in the data to calculate both parameters with confidence. Therefore, to obtain a representative value for the viscosity at a given temperature, a single value for the critical force was used to calculate the viscosity for each cell at a given temperature by single parameter least squares. These values were then averaged to obtain the mean value for the viscosity at that temperature.

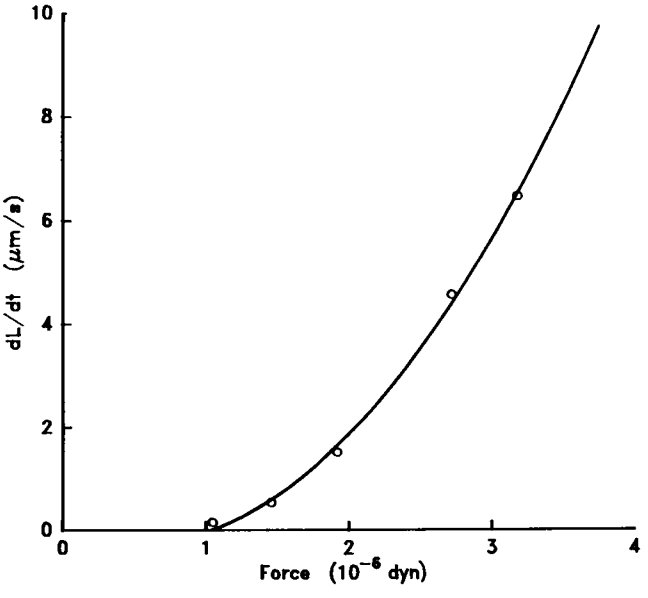

FIGURE 1 The solid line shows the theoretical prediction for tether growth rate as a function of force for given values of the critical force and viscosity. Points represent data for a cell at 40°C. The values of the critical force, 1.05×10^{-6} dyn, and the viscosity coefficient, 6.7×10^{-4} dyn s/cm, were determined by least squares.

TABLE I
BEST FIT DETERMINATIONS OF THE CRITICAL FORCE AT EACH TEMPERATURE

Temperature	Average	SD	n
(°C)	$F_c(10^{-6}$ dyn)		
12.0	1.11	0.54	5
16.5	1.01	0.42	12
25.0	1.48*	0.32	8
34.6	1.14	0.23	9
40.0	1.05	0.21	8

*A previous study found a value of 1.05 ± 0.11 (n = 16) at 21°C (Waugh and La Celle, 1980)

TABLE II
PLASTIC VISCOSITY COEFFICIENT AT EACH TEMPERATURE

Temperature	Average	SD	n
(°C)	η_p(dyn s/cm)		
12.0	0.029	0.012	18
16.5	0.0101	0.0037	18
25.0	0.0072	0.0046	17
34.5	0.0027	0.0019	33
40.0	0.00126	0.00062	15

RESULTS

Measurements were performed at six temperatures, but results at the lowest temperatures (<7.0°C) were inconsistent, so most of the data were obtained in the temperature range 10.0–40.0°C. Even in this range the scatter in the data was large, but it was typical of flow channel measurements. About 40% of the cells behaved with enough consistency to determine both the critical force and the surface viscosity by least squares.[2] The data for these "best fits" are summarized in Table I. The critical force did not change significantly over the experimental temperature range. All of the data are consistent with a critical force of 1.0×10^{-6} dynes, in precise agreement with previously published values (Hochmuth et al., 1976; Waugh and La Celle, 1980). To make reliable comparisons among the viscosities at different temperatures, a value for the critical force of 1.0×10^{-6} dyn was used in all calculations of viscosity.

The distribution of viscosity values at each temperature was positively skewed. For example, Fig. 2 shows the distribution of viscosities at 12.0°C. To avoid an inappropriate shift in the mean due to a few extremely large values, viscosities that were more than two standard deviations from the mean (of all cells at a given temperature) were disregarded. The arrows in Fig. 2 show the cut-off value and the mean of remaining distribution at 12.0°C. The means and standard deviations of the populations below the cut-off at each temperature are given in Table II. The change in the viscosity coefficient with temperature was exponential. Fig. 3 shows the natural log of the viscosity plotted against inverse temperature. The relationship appears to be linear over the experimental temperature range.

Measurements <7.0°C were difficult to obtain because the viscosity was very large, and the forces needed to produce large tether growth rates could not be supported by the tether. Either the tether broke, or it was pulled loose at the attachment site. The few measurements that were obtained at 6.5°C were consistent with a critical force of 1.0×10^{-6} dyn and showed viscosities between 0.02 and 0.07 SP (dyn s/cm). These values span the viscosity value predicted by extrapolating the curve in Fig. 3 to 6.5°C.

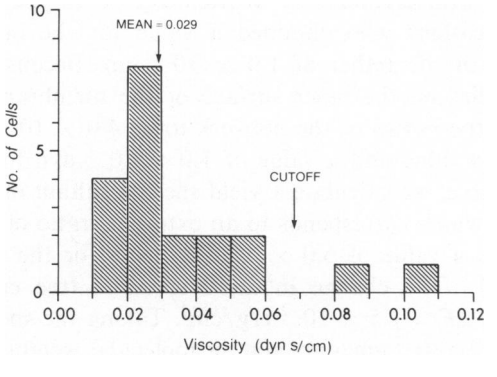

FIGURE 2 Distribution of viscosities at 12°C. The arrow at the right shows the cutoff value. Viscosities greater than that value were discarded. The arrow at the left shows the mean of the remaining population.

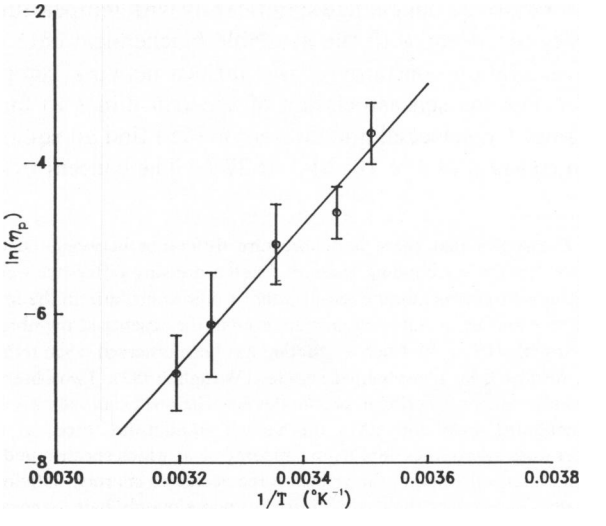

FIGURE 3 Natural log of the plastic viscosity coefficient as a function of inverse temperature. The slope of the line was determined by least squares and gives a temperature-viscosity coefficient of 18.6 kcal/mol.

[2]Data from the remaining cells were consistent with data from these "well-behaved" cells (for a given force on the cells the tether growth rates were similar), but either there were not enough data pairs or the scatter in the data was too large to determine two parameters for the individual cell. There was no evidence of physical differences among the cells, there was simply not enough resolution in the data to perform reliable calculations of the critical force.

DISCUSSION

The critical force is an experimental parameter, that is, it is not an intrinsic property of the material. It is related to the yield shear resultant (an intrinsic material property) by

$$\hat{T}_s = \frac{F_c}{4\pi r_t} \tag{6}$$

where r_t is the radius of the tether[3] (Evans and Hochmuth, 1976). Some measurement of the tether radius is needed to calculate the yield shear resultant. In an unpublished study, Wattenberg, Hochmuth, and Williamson have measured tether diameters by fixing the cells in glutaraldehyde and examining them in an electron microscope. At a constant applied shear stress the change in the tether diameter with temperature was <10.0% over the range 15–45°C. Based on these measurements and our own measurements of the critical force, we conclude that the yield shear resultant does not change appreciably with temperature.

At a molecular level the yielding of the membrane corresponds to a breakdown in the protein network that gives the membrane its shear rigidity, possibly due to the rupture of intermolecular bonds. The major proteins of this network have been identified biochemically. Spectrin, actin, and band 4.1 appear to make up the major framework of the network. The manner in which they are connected is the subject of some controversy, but it seems apparent from the experiments of Ungewickell and Gratzer (1978) and the work of Ungewickell et al. (1979) that dimer-dimer association of spectrin and the spectrin-4.1-actin complex are important connections within the network. The rupture of either of these connections would result in the loss of membrane shear rigidity.

The fact that the yield shear resultant is not a strong function of temperature indicates that the number and strength of the intermolecular connections within the network also do not change significantly with temperature. This is consistent with the available biochemical data on the association constants of the various network components. For the self-association of spectrin dimer to form tetramer Ungewickell and Gratzer (1978) find an equilibrium constant of $3 \times 10^5\ M^{-1}$ at 37°C. The concentration of spectrin on the surface is $\sim 1.0 \times 10^{-7}\ g/cm^2$ (Fairbanks et al., 1971), and the molecular weight of the tetramer is ~900,000. Assuming the thickness of the network is on the order of 4.0 nm, we can calculate a spectrin concentration on the membrane of ~250 mg/ml, or 0.3 mM. At this concentration we would predict that ~10.0% of the spectrin would be in dimeric form at 37.0°C. Because spectrin tends to become more associated at lower temperature, the biochemical data predict that the number of spectrin-spectrin associations should decrease by <10.0% over the experimental temperature range. Recently, Sheetz and co-workers (personal communication) have obtained a value of the association constant of the spectrin-4.1-actin complex. Using their value for the association constant of $1.4 \times 10^7\ M^{-1}$ (37°C) and making a similar calculation, we predict that <2.0% of the protein would exist in the dissociated form at 37°C. Thus, based on biochemical evidence, we expect the number of connections within the network to change by <12% over the experimental temperature range. Such a small change would not be detected in our measurement of the yield shear resultant.

It is possible to estimate the free energy change in the network due to the work of shear deformation which results in material failure. Assuming that the shear modulus is constant throughout the elastic range, the change in the Helmholtz free energy density at a material point, $\Delta\tilde{F}$, is given by (Evans and Waugh, 1977) $\Delta\tilde{F} = \mu\beta$ where μ is the surface elastic shear modulus, and β is the shear deformation parameter. At constant area, $\beta = (1/2)(\lambda^2 + \lambda^{-2} - 2)$, where λ is the extension in the surface. The extension at yield can be calculated from the yield shear resultant via the elastic constitutive equation (Eq. 3). The yield shear resultant is obtained from our measurement of the critical force and Eq. 6. The value for the tether radius is obtained from the measurements of Wattenberg et al. (personal communication) who obtained a value for the outside diameter of the tether of 1.0×10^{-5} cm. Because the network lies on the inner surface of the membrane we estimate the radius of the network to be 4.0×10^{-6} cm. Using this value and a value of 1.0×10^{-6} dyn for the critical force, we calculate a yield shear resultant of 0.02 dyn/cm, which corresponds to an extension ratio of 2.6. (We used a value of 6.0×10^{-3} dyn/cm for the shear modulus.) The change in the Helmholtz free energy density is $\Delta\tilde{F} \simeq 1.5 \times 10^{-2}\ erg/cm^2$. Taking the spectrin density to be $10^{-7}\ gm/cm^2$, and the molecular weight to be 900,000 (tetramer) we find the change in free energy per mole of tetramer to be: $\Delta F \simeq 3.2$ kcal/mol.

The exponential dependence of viscosity on temperature is typical of most liquids. It can be expressed as η = constant $e^{-E/RT}$, where η is viscosity, E is the temperature-viscosity coefficient and RT is the product of the gas constant and absolute temperature. For simple liquids, E is constant over wide temperature ranges. Eyring originally identified this quantity as the activation energy for the translocation of a molecule (or molecular segment) within

[3]Eq. 6 assumes that there is no pressure difference between the cell interior and the surrounding medium. Such a pressure difference would result in isotropic membrane tensions that would contribute to the force balance, resulting in static equilibrium even in the absence of membrane shear rigidity ($\hat{T}_s = 0$). Such a situation has been observed when tethers were formed from phospholipid vesicles (Waugh, 1982). Two observations indicate that the critical force in the flow channel experiments is due to membrane yield and not a mechanical equilibrium. First, as the temperature approaches 48°C (the temperature at which spectrin undergoes a structural change) the critical force decreases sharply. (Preliminary data shows that the critical force is approximately half its normal value at 46°C.) Second, in cells with a membrane structural defect, the critical force is approximately one-third of normal (Waugh and LaCelle, 1980).

the flowing liquid. This interpretation is not valid for polymers because the translocation of a polymer segment depends not only on the energy available to the segment, but also the conformation of neighboring chains and the number of entanglements in the network. Because of the additional constraints of network geometry, the true activation energy for molecular dislocation in a polymer is less than or equal to the temperature-viscosity coefficient. In general, however, the coefficient, E, can be regarded as a measure of the ease with which a molecular segment can jump to its next location within the flowing network.

With this interpretation in mind, it is interesting to compare temperature-viscosity coefficients of different polymers with the coefficient for erythrocyte membrane. A few of these are summarized in Table III. Two coefficients are given for the erythrocyte membrane. One is obtained from the data of the present study for the membrane viscosity above the elastic limit, the plastic viscosity coefficient. The other is calculated from the data of Hochmuth et al. (1980) on the temperature dependence of the viscoelastic recovery of the erythrocyte membrane, the viscoelastic viscosity coefficient, i.e., the viscosity of the membrane below the elastic limit. The coefficients for erythrocyte membrane are comparable to the coefficients of other polymeric materials. The fact that the coefficient for viscoelastic deformation is smaller than the coefficient for plastic deformation of the membrane is consistent with the notion that the molecular rearrangements within the network below the elastic limit are less extensive than those that occur in plastic flow.

The magnitude of these mechanically determined quantities ought to be comparable to the biochemically determined activation energies for the corresponding dissociation of network components. Both the work required to initiate flow (~3.2 kcal/mol) and the activation energy needed to sustain flow (<19 kcal/mol) are considerably less than the activation energy for spectrin dissociation (110 kcal/mol) (Ungewickell and Gratzer, 1978). The possibility that the activation energy for this dissociation is lowered drastically by the conformational changes that occur in deformation seems unlikely. Therefore, we conclude that the spectrin-spectrin connection is not broken when the membrane yields, but that some other molecular connection within the network is involved. The spectrin-4.1-actin linkage is a possible candidate, but preliminary data of Sheetz shows a strong temperature dependence for the rate of skeletal dissociation, indicating that the activation energy for that process is also quite high. These observations have led us to speculate about the nature of the molecular events that occur when the membrane is deformed (See Appendix).

TABLE III
TEMPERATURE-VISCOSITY COEFFICIENTS

Material	E	Source
	(kcal/mole)	
RBC membrane (elastic)	10.0	Hochmuth, et al., 1980
RBC membrane (plastic)	18.6	Waugh, 1982
Natural Rubber	10.1	Ewell, 1938
Polyester	8.3	Flory, 1940
Polyisobutylene	21.1	Ferry and Parks, 1935

Although the model presented in the Appendix is purely speculative there are several substantive points to be made. Based on the stoichiometry and surface density of network components the expected distance between molecular connections on the membrane is much less than the extended length of the spectrin molecule. Furthermore, the ratio of the interjunction distance to the extended molecular length is nearly equal to the estimated material extension at which the membrane yields. Finally, because the mechanical work needed to overcome the elastic limit of the membrane is apparently much less than the energy required to break either spectrin-spectrin bonds or the spectrin-4.1-actin connection, we propose that these connections remain largely intact when the membrane yields. Further experiments will be required to draw more specific conclusions about the molecular events which account for membrane failure in shear deformation.

APPENDIX

We consider the network as an array of junctions that are connected by springs. The junctions correspond to short actin filaments (protofilaments), and the springs correspond to spectrin tetramers in keeping with the network organization described by Lux (1979). We would like to compare the average distance between junctions to the length of the spectrin molecule. To calculate the interjunction distance, let us suppose that the junctions are arranged in a close-packed hexagonal array, as shown in Fig. 4. Each junction is surrounded by six triangular areas which are each bounded by three springs. In this configuration there are three springs per junction and one junction for every two triangular areas. Because the density of spectrin is $\sim 10^{-7}$ g/cm^2 and the molecular weight of the tetramer is 9×10^5, we calculate that there are 6×10^{10} springs/cm^2 on the surface. Because there are three springs per junction there are 2×10^{10} junctions/cm^2, and because there are two triangular areas for each junction there are 4×10^{10} triangles/cm^2. Thus,the area of each triangle is 25×10^{-12} cm^2, which means that the length of each side is 7.6×10^{-6} cm (76 nm). This is the distance between junctions.[4] It is much less than the length of an extended spectrin tetramer (200 nm) as determined from electron micrographs (Shotten et al., 1979).

The model is consistent with existing data, both biochemical and mechanical. There are 4–5 copies of actin for each spectrin tetramer on the membrane (Lux, 1979; Gratzer, 1981), but the number of actins per protofilament is 12–18 (Gratzer, 1981, Brenner and Korn, 1980). This makes the ratio of tetramer:protofilament ~3:1, which is precisely the ratio of springs:junctions in our model. (Other arrays could be used to calculate the interjunction distance, but the spring to junction ratio would not agree with the biochemical data.) Finally the ratio of the unstressed spring length (76 nm) to the fully extended length of a spectrin tetramer (200 nm) is 2.6, which is very close to the material extension ratio at which the membrane loses its elastic character.

[4]A similar distance can be calculated without resorting to an ordered array. Since the ratio of tetramer to protofilaments is 3:1, and the density of tetramer is 6×10^{10} molecules/cm^2, the area per junction is 5×10^{-11} cm^2. A characteristic distance between junctions can be found by taking the square root of that area: 71 nm.

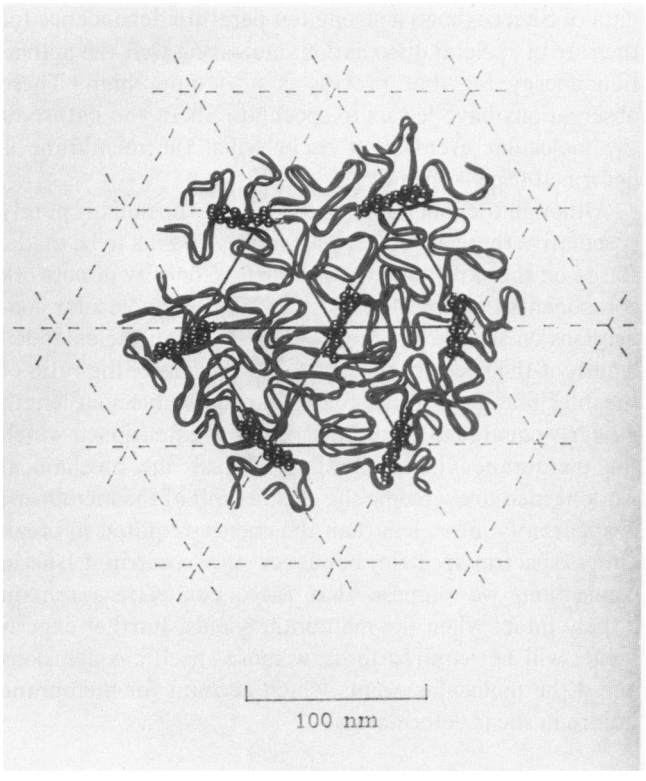

FIGURE 4 Hypothetical organization of the membrane skeletal network. Thick lines represent spectrin tetramers, spheres represent actin protofilaments. The ratio of the length of the tetramer to the interfilament distance is 2.6.

This simple calculation suggests the following series of molecular events during deformation. In the unstressed state spectrin exists in a coiled conformation, such that the end to end distance is ~76 nm. As the membrane is deformed the end to end distance changes, but the conformational change is reversible as long as the end to end distance does not exceed the natural length of the molecule (200 nm). When the molecule approaches its fully extended length the mechanical forces rupture intramolecular bonds within the spectrin molecule. As successive bonds are broken under constant mechanical force the molecular length continues to increase and the membrane flows. When the force is released the spectrin gradually refolds, and the tether is reabsorbed into the body of the cell. (Tether resorption is observed experimentally.)

The author thanks Dr. Michael Sheetz for his helpful discussion of changes in molecular equilibrium of the cytoskeleton with temperature, and for the use of his data prior to publication. The author thanks Dr. Joseph Williamson and Dr. Robert Hochmuth for providing access to their unpublished measurements. The author also recognizes Mrs. Donna Brooks for her technical assistance.

Support for this work was obtained under National Institutes of Health grant HL 26485-01. Additional support was obtained under contract with the U.S. Department of Energy at the University of Rochester Department of Radiation Biology and Biophysics. This paper has been assigned Report No. UR-3490-2100.

Received for publication 30 November 1981 and in revised form 5 April 1982.

REFERENCES

Brenner, S. L., and E. D. Korn. 1980. Spectrin/actin complex isolated from sheep erythrocytes accelerates actrin polymerization by simple nucleation. *J. Biol. Chem.* 255:1670–1676.

Evans, E. A., and R. M. Hochmuth. 1976. Membrane viscoplastic flow. *Biophys. J.* 16:13–26.

Evans, E. A., and R. Waugh. 1977. Mechano-chemistry of closed vesicular membrane systems. *J. Colloid Interface Sci.* 60:286–298.

Ewell, R. H. 1938. The reaction rate theory of viscosity and some of its applications. *J. Appl. Phys.* 9:252–269.

Fairbanks, G., T. L. Steck, and D. F. H. Wallach. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry.* 10:2606–2617.

Ferry, J. D., and G. S. Parks. 1935. Viscous properties of polyisobutylene. *Physics (New York).* 6:356–362.

Flory, P. J. 1940. Viscosities of linear polyesters. An exact relationship between viscosity and chain length. *J. Amer. Chem. Soc.* 62:1057–1070.

Gratzer, W. B. 1981. The erythrocyte membrane and its cytoskeleton. *Biochem. J.* 198:1–8.

Hochmuth, R. M., K. L. Buxbaum, and E. A. Evans. 1980. Temperature dependence of the viscoelastic recovery of erythrocyte membrane. *Biophys. J.* 29:177–182.

Hochmuth, R. M., E. A. Evans, and D. F. Colvard. 1976. Viscosity of human red cell membrane in plastic flow. *Microvasc. Res.* 11:155–159.

Hochmuth, R. M., N. Mohandas, and P. L. Blackshear, Jr. 1973. Measurement of the elastic modulus for erythrocyte membrane using a fluid mechanical technique. *Biophys. J.* 13:747–762.

Lux, S. E. 1979. Dissecting the erythrocyte membrane cytoskeleton. *Nature (Lond.).* 281:426–429.

Shotten, D., B. Burke, and D. Branton. 1979. The molecular structure of human erythrocyte spectrin: biophysical and electron microscopic studies. *J. Mol. Biol.* 131:303–329.

Ungewickell, E., P. M. Bennett, R. Calvert, V. Ohanian, and W. B. Gratzer. 1979. *In vitro* formation of a complex between cytoskeletal proteins of the human erythrocyte. *Nature (Lond.).* 280:811–814.

Ungewickell, E., and W. Gratzer. 1978. Self-association of human spectrin: a thermodynamic and kinetic study. *Eur. J. Biochem.* 88:379–385.

Waugh, R. E. 1982. Surface viscosity measurements from large bilayer vesicle tether formation. I. Analysis. *Biophys. J.* 38:19–27.

Waugh, R. E., and E. A. Evans. 1979. Thermoelasticity of red blood cell membrane. *Biophys. J.* 26:115–131.

Waugh, R. E., and P. L. La Celle. 1980. Abnormalities in the membrane material properties of hereditary spherocytes. *J. Biomech. Engr.* 102:240–246.