BRIEF COMMUNICATION

TEMPERATURE DEPENDENCE OF THE VISCOELASTIC RECOVERY OF RED CELL MEMBRANE

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ABSTRACT The time-dependent recovery of an elongated red cell is studied as a function of temperature. Before release, the elongated cell is in static equilibrium where external forces are balanced by surface elastic force resultants. Upon release, the cell recovers its initial shape with a time-dependent exponential behavior characteristic of a viscoelastic solid material undergoing large ("finite") deformation. The recovery process is characterized by a time constant, t_c, that decreases from ~0.27 s at 6°C to 0.06 s at 37°C. From this measurement of the time constant and an independent measurement of the shear modulus of surface elasticity for red cell membrane, the value for the membrane surface viscosity as a function of temperature can be calculated.

Recently, Hochmuth et al. (1979) measured the time-dependent recovery at 25°C of a human red blood cell that is extended by pulling at diametrically opposite points on the cell rim (Fig. 1). The recovery process is driven by the elastic forces in the red cell membrane. The rate of recovery is limited by the viscous dissipation in the red cell membrane and adjacent fluid phases. As we show in this brief communication, the recovery process can be characterized by a time constant, t_c, that decreases from ~0.27 s at 6°C to 0.06 s at 37°C. For a time constant >0.01 s, the viscous dissipation in the adjacent cytoplasmic and extracellular fluid phases is negligible compared with the elastic "power" of the membrane; therefore, the elastic power is dissipated by viscous processes in the membrane itself. A first-order theory of membrane viscoelasticity based on parallel superposition of elastic and viscous processes in the membrane provides a coefficient of membrane surface viscosity, η, that is given by the product of the membrane elastic shear modulus, μ, and the measured time constant. Thus, the static measurement of the elastic shear modulus (Evans, 1973; Waugh and Evans, 1979) and the temporal observation of membrane extensional recovery as a function of temperature can be combined to determine the coefficient of membrane surface viscosity as a function of temperature, since $\eta = t_c \cdot \mu$. This coefficient characterizes viscous dissipation in the membrane when the membrane responds as a solidlike material.

Blood samples were obtained from healthy, adult donors, with heparin as the anticoagulant. Cells were washed three times in phosphate-buffered saline (121.5 mM NaCl, 25.2 mM Na_2HPO_4, and 4.8 mM KH_2PO_4; pH was adjusted to 7.4 at 25°C and osmolarity adjusted to 300 mOsM) and, after the final wash, resuspended in phosphate-buffered saline plus 0.1% human serum albumin. The washed cells were infused into a chamber formed by a glass slide,

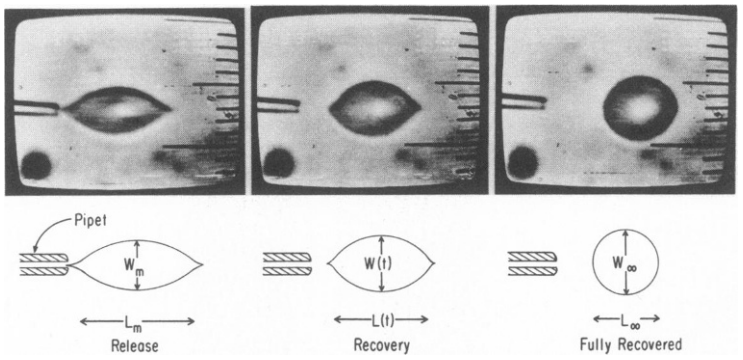

FIGURE 1 Cell outlines and photographs that illustrate the extensional recovery of a single red cell at 15°C. During the process, both length and width are measured as a function of time. The length, L_m, and width, W_m, are measured on the first scan after release (i.e., release + 1/60 s). The middle photograph was taken at 0.12 s after release. The cell was attached at a single point to the bottom surface of a temperature-controlled chamber and viewed in monochromatic light (439 nm) with an inverted microscope and long-working-distance objective (40X, 0.60 numerical aperature). The photographs were taken from video recordings of a single-cell experiment.

gasket, and coverslip, and the chamber was placed on the stage of an inverted microscope. The cells in the chamber settled and subsequently attached to the lower (coverslip) surface. Infusion of an 8% native plasma solution in phosphate-buffered saline quenched the attachment process. The temperature in the sample chamber was controlled by an adjacent water-jacketed heat exchanger, and measured with a thermocouple. Cells with "single-point" attachments were selected; then deformation was produced by aspirating the rim of a cell into a micropipet at a location opposite to the point of attachment to the coverslip. Recovery (Fig. 1) occurred when the rim of the cell was released from the pipet. The recovery process was recorded on video tape at 60 "scans"/s; thus, cell length and width measurements were made every 1/60 s.

Evans and Hochmuth (1976, 1978) have shown that the first-order theory for superposition of elastic and viscous processes in the membrane provides the following expression for the maximum shear resultant (maximum shear force per unit length in the surface), T_s:

$$T_s = \frac{\mu}{2}(\lambda^2 - \lambda^{-2}) + 2\eta \cdot \frac{1}{\lambda}\frac{d\lambda}{dt}, \tag{1}$$

where λ is the membrane extension ratio, t is time, μ is the shear modulus of surface elasticity, and η is the coefficient of surface viscosity. Since the membrane surface area remains constant during extension (Evans, 1973; Evans and Hochmuth, 1976, 1978), the principal extension ratio in the direction of elongation is given by the square root of the length-to-width ratio of an element of the membrane surface (Evans, 1973). Both the elastic and viscous terms in Eq. 1 are nonlinear in the extension ratio, λ, because large ("finite") deformations of the membrane are involved.

If the surface were shaped like a rectangle, the instantaneous length-to-width ratio, L/W,

relative to some undeformed length-to-width ratio, $(L/W)_\infty$, would be the measure of λ^2.[1] Thus, we introduce the following expression for the square of the extension ratio:

$$\lambda^2 = (L/W)/(L/W)_\infty. \tag{2}$$

During the recovery process, $T_s \simeq 0$ when dissipation in the extracellular and cytoplasmic fluids is negligible. Therefore, we substitute Eq. 2 into Eq. 1 and integrate to obtain the instantaneous length-to-width ratio for an initial (maximum) length-to-width ratio of $(L/W)_m$:

$$\frac{L}{W} = \frac{\Lambda + \exp(-t/t_c)}{\Lambda - \exp(-t/t_c)}, \tag{3}$$

where

$$\Lambda \equiv \frac{(L/W)_m + (L/W)_\infty}{(L/W)_m - (L/W)_\infty},$$

and

$$t_c \equiv \eta/\mu. \tag{4}$$

Eq. 4 defines the characteristic recovery time constant, t_c, in terms of the viscous and elastic membrane material properties, η and μ. Eq. 3 represents the local time-dependent recovery of an element of the membrane surface. Although the membrane extension is not uniform over the cell surface, we have shown (Hochmuth et al., 1979) that the measured aspect ratio of cell length-to-width in Eq. 3 is a good approximation to the whole-cell behavior. In Eq. 3, values for $(L/W)_\infty$ and t_c are chosen to give a best (least-squares) fit to the experimental data (Fig. 2).[2]

It is interesting to note that Eq. 3 is easily rearranged so that its linear behavior on a semilogarithmic plot is demonstrated:

$$\ln\left[\frac{\left(\frac{L}{W}\right) + \left(\frac{L}{W}\right)_\infty}{\left(\frac{L}{W}\right) + \left(\frac{L}{W}\right)_\infty} \cdot \frac{\left(\frac{L}{W}\right)_m - \left(\frac{L}{W}\right)_\infty}{\left(\frac{L}{W}\right) - \left(\frac{L}{W}\right)_\infty}\right] = \frac{t}{t_c}. \tag{5}$$

In fact, in our preliminary studies of the recovery process at 25°C, we plotted the data in this fashion (Hochmuth et al., 1978). However, correlation of the data with a logarithmic

[1] A better approximation to the actual shape of the membrane surface would be a flat circular disk in the undeformed state. However, we have shown analytically that both a disk (with a nonuniform extension over the surface) and a rectangular strip (with a uniform extension) recover at nearly the same rate, with the initial rate of recovery of the disk being somewhat faster than that of the strip (Hochmuth et al., 1979).

[2] In Fig. 2 we note that the experimental data for short times ($t \leq t_c$) indicates greater (e.g., "faster") recovery than the theoretical curve generated by the "strip approximation." This is consistent with our earlier observations (Fig. 8; Hochmuth et al. 1979). As we showed theoretically at that time, a disklike body will always recover faster initially than a strip because of the larger extension ratios and, thus, higher elastic energy densities in the end regions of the disk.

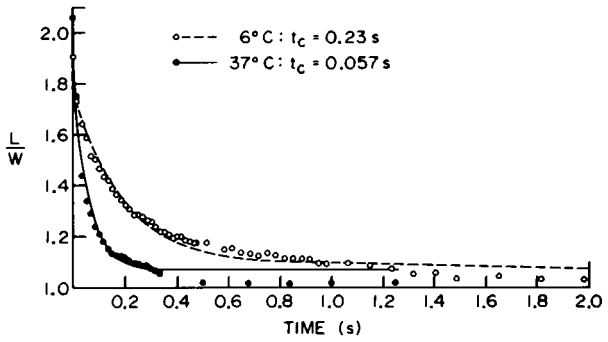

FIGURE 2 Experimental and theoretical results given in terms of the length-to-width ratio for two cells, one of which was at 6°C (open circles and dashed line) while the other was at 37°C (solid circles and solid line). Eq. 3 is fitted to the data shown in this figure with values for the two parameters, $(L/W)_\infty$ and t_c, chosen to minimize the sum of the squares of the errors between the viscoelastic model (Eq. 3) and experimental values for L/W. Thus, for N data points the following functional is minimized by choosing optimum values for $(L/W)_\infty$ and t_c:

$$\epsilon^2 = \sum_1^N \left[\left(\frac{L}{W}\right)_{\text{expr}} - \left(\frac{L}{W}\right)_{\text{theo}}\right]^2 / N,$$

where ϵ is the root mean square error per datum point. For the two sets of results shown in this figure, the value of ϵ is $\sim$ 0.03. This error corresponds to errors in length and width measurements of $\sim$ 0.1 μm. Thus, the errors are within the limits of optical resolution of the measuring system. The experimental data correlates closely with the theory (Eq. 3) for times less than approximately four recovery time constants (1.4 s at 6°C and 0.36 s at 37°C).

expression like Eq. 5 results in an undue emphasis on the data for long time periods where $L/W \rightarrow (L/W)_\infty$ and where our planar viscoelastic approximation is no longer valid because of the subtle effects of "bending" (curvature elastic energy) and the permanent (plastic) flow that can occur in the membrane, especially at the tips, before release. With the two-parameter $[(L/W)_\infty, t_c]$, least-squares minimization of the functional given by Eq. 3, we can correlate the data over a range of approximately four time constants. Thus, at 6°C, the range over which Eq. 3 is applicable is $0 \leq t \lesssim 1.0$ s, while at 37°C, $0 \leq t \lesssim 0.25$ s (Fig. 2).

During the recovery process, it is clear that individual experimental data points (Fig. 2) deviate from the "best fit" of Eq. 3. However, these deviations are within the experimental accuracy. For example, for a length L of 9 μm and a width W of 7.5 μm,

$$\frac{L}{W} = 1.2 \pm 0.05, \tag{6}$$

for an uncertainty in the optical measurements of 0.2 μm. Maximum deviations shown on Fig. 2 for $0 \geq t \lesssim 4\, t_c$ are well within this range.

In the red cell extension and recovery experiments, we have observed (Fig. 1) that the projected cell area remains essentially constant throughout the experiment. Since the interior volume is constant, the mean cell thickness, δ, is essentially constant during the experiment. This implies that fluid layers between the upper and lower cell surfaces experience deformation that is geometrically similar to the cell projection. Therefore, the rate of shear deformation in the cell interior is approximately the same as the rate of shear deformation in

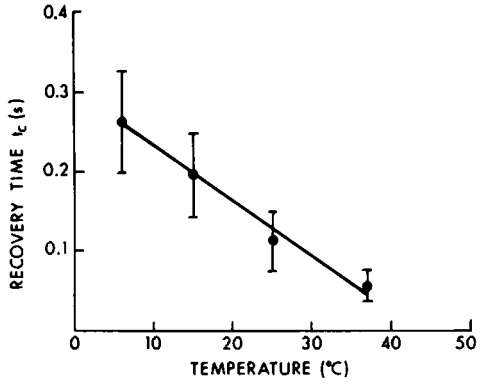

FIGURE 3 The recovery time constant at four different temperatures determined according to the method outlined in Fig. 2. The numbers of experiments performed (cells studied) at each temperature were 34(6°C), 34(15°C), 36(25°C), and 47(37°C). The error bars represents a linear best-fit to the experimental data points.

the membrane surface. With this approximation (Evans and Hochmuth, 1976; Hochmuth et al., 1979), we can estimate the ratio of mechanical power dissipated in the cytoplasm, $\dot{W}_{Hb}$, to the elastic "power" of the upper and lower cell membrane surfaces, $\dot{W}_m$, as

$$\left| \frac{\dot{W}_{Hb}}{\dot{W}_m} \right| \approx \frac{\tilde{\eta}_{Hb}\delta}{2(t_c\mu)}, \tag{7}$$

where $\tilde{\eta}_{Hb}$ is the (three-dimensional) viscosity of the hemoglobin solution. At room temperature, $t_c \approx 0.1$ s (Fig. 3), $\mu \approx 10^{-2}$ dyn/cm (Evans, 1973; Waugh and Evans, 1979), and $\tilde{\eta}_{Hb} \approx 10^{-1}$ dyn · s/cm^2 (Cokelet and Meiselman, 1968). Thus,

$$\frac{\dot{W}_{Hb}}{\dot{W}_m} \approx 0.01 \tag{8}$$

for $\delta = 2 \times 10^{-4}$ cm. Consequently, viscous dissipation in the membrane limits the rate of elastic recovery. This ratio remains small throughout the temperature range under investiga-

TABLE I
CALCULATION OF THE SURFACE VISCOSITY

Temperature	t_c	$(\mu \times 10^3)$*	$(\eta \times 10^4)$
(°C)	(s)	(dyn/cm)	(dyn·s/cm)
6	0.273	7.8	21
15	0.200	7.2	14
25	0.111	6.7	7.4
37	0.063	5.7	3.6

*Data from Waugh and Evans (1979).
Calculation of the surface viscosity, η, from measurements of the recovery time constant, t_c (Fig. 3), and the shear modulus of surface elasticity, μ; i.e., $\eta = t_c\mu$. A surface viscosity of 7.4×10^{-4} poise-cm (dyn · s/cm, poise · cm, or "surface poise") is 0.74×10^{-4} N · s/m in the international system of units.

tion. (The surface elasticity, μ, changes only slightly with temperature [Waugh and Evans, 1979] and our own measurements have indicated that the viscosity of concentrated hemoglobin solutions [30 gm/100 ml] is $\sim 1.4 \times 10^{-1}$ dyn · s/cm^2 at 6° and 0.6×10^{-1} dyn · s/cm^2 at 35°C.) Thus, dissipative effects within the cytoplasm are negligible compared with membrane dissipation over the entire temperature range. In addition, the extracellular fluid appears to have no effect on the recovery process since the viscosity of the extracellular fluid is $\sim 1/10$ that of hemoglobin. Also, if the extracellular fluid retarded the recovery process, then the cell shape would be asymmetric (teardrop shape) during the recovery phase. Clearly, this is not the case (Fig. 1).

With values for t_c as a function of temperature (Fig. 3) and μ as a function of temperature (Waugh and Evans, 1979), values for η are readily calculated (Table I). The membrane surface viscosity, η, decreases as a function of temperature. From Table I, we estimate that in the range between 6 and 37°C, the viscosity decreases by almost a factor of 6.

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