MEMBRANE VISCOELASTICITY

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ABSTRACT   In this paper, we develop a theory for viscoelastic behavior of large membrane deformations and apply the analysis to the relaxation of projections produced by small micropipette aspiration of red cell discocytes. We show that this relaxation is dominated by the membrane viscosity and that the cytoplasmic and extracellular fluid flow have negligible influence on the relaxation time and can be neglected. From preliminary data, we estimate the total membrane "viscosity" when the membrane material behaves in an elastic solid manner. The total membrane viscosity is calculated to be 10^{-3} dyn-s/cm, which is a surface viscosity that is about *three orders* of magnitude *greater* than the surface viscosity of lipid membrane components (as determined by "fluidity" measurements). It is apparent that the lipid bilayer contributes little to the fluid dynamic behavior of the whole plasma membrane and that a structural matrix dominates the viscous dissipation. However, we show that viscous flow in the membrane is not responsible for the temporal dependence of the isotropic membrane tension required to produce lysis and that the previous estimates of Rand, Katchalsky, et al., for "viscosity" are *six* to *eight orders* of magnitude too large.

INTRODUCTION

Recoverable deformations in plasma membranes have been observed and studied since the beginning of microscopic observation of cell morphology (Ponder, 1971). Reversible (elastic) shape changes of plasma membranes are especially apparent in the flow of red blood cells through the microcirculation of vertebrate animals (Krogh, 1930). In this case, the red cell is forced to negotiate channels and apertures significantly smaller than its major dimension, creating large membrane deformations. As reported by Krogh, it does so with ease and resumes its initial discoid shape after leaving the constriction. The normal mammalian, red blood cell membrane must support all of the equilibrium forces responsible for deformation because the interior is a homogeneous liquid. Therefore, along with its geometric simplicity, the mammalian red blood cell has provided a convenient system for the study of membrane elasticity.

Direct mechanical experiments on single, human red blood cells were first performed by Rand and Burton (1964) by aspirating cells into a micropipette. Since then, these experiments have been expanded (LaCelle, 1969, 1970, 1972; Leblond, 1972) and both normal and diseased red cells have been studied extensively. In addition, another experimental technique involving the fluid shear deformation of red cells attached to

glass substrates was used to investigate the hyperelastic behavior of the red cell (Hochmuth and Mohandas, 1972; Hochmuth et al., 1973). These experiments showed that the red cell membrane was capable of large elastic deformations ("stretch") provided the membrane area was not required to increase. Recently, an innovative, two-dimensional hyperelastic material concept and finite deformation analysis (Skalak et al., 1973; Evans, 1973 a, b) provided correlation with the mechanical experiments and accounted for the large resistance to area increase observed in osmotic swelling of red cells.

Significantly less attention has been given to the time response of the membrane tó applied forces. Viscoelastic behavior of red cells has been considered by Rand (1964) for lysis in a relatively large micropipette (ca. 3 μm) and Katchalsky et al. (1960) in the analysis of osmotic lysis. However, as we will subsequently show, viscous flow in the membrane is not responsible for the temporal dependence of the membrane tension required to produce lysis[1] because the rate of deformation in this case is very small. Viscoelastic relaxation has been observed after cells have been rapidly expelled from relatively large micropipettes (Hoeber and Hochmuth, 1970). However, no quantitative determination has been made of the intrinsic material properties that characterize the relaxation of large membrane deformations.

In this paper, we present a simple theory for viscoelastic behavior of large membrane deformations and apply the analysis to the relaxation of projections produced by small micropipette aspiration of red cell discocytes. From preliminary data, we estimate the total membrane "viscosity" when the membrane material behaves in an elastic solid manner.[2] We show that this relaxation is dominated by the membrane viscosity and that the cytoplasmic and extracellular fluid flow have negligible influence on the relaxation time and can be neglected.

TWO-DIMENSIONAL VISCOELASTICITY

In previous developments (Skalak et al., 1973; Evans, 1973 a, b, 1975 b), it has been shown that the red cell membrane behaves as a two-dimensional, anisotropic material (but isotropic in the plane of the membrane). Stresses in the plane of the membrane are not coupled to the direction normal to the surface,[3] i.e., the membrane cannot change thickness in response to an in-plane stress but can only change the shape of a

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[1]The time dependence of isotropic tension at lysis is probably a phenomenon of relaxation of internal forces concentrated at rigid "cross-link" points of a structural matrix (Evans, 1975 a). The isotropic tension is initially supported by the matrix, but relaxes with time until the lipid bilayer is required to increase area. The lipid bilayer can only support low tensions compared with the initial tensions reported by Rand (1964) of 20 dyn/cm. Also, the surface area can only increase by a small percentage before lysis occurs. The relaxation of isotropic tension, however, may involve large molecular displacements in the structural matrix but the ensemble average of these displacements results in a negligible material deformation (area increase). Therefore, a continuum flow model (e.g., simple viscoelasticity for failure of a membrane in isotropic tension) would be inappropriate.

[2]In a companion article, we consider viscous material behavior of the membrane after plastic failure.

[3]Normal surface traction differences (e.g., hydrostatic pressure difference) must be opposed by resultant components that arise from surface curvature.

surface element. From the ultrastructural viewpoint, this is expected because the material is a composite of molecular monolayers: continua in two-dimensions with molecular character in the third. Because of the fixed thickness, applied forces are considered to be distributed per unit length (resultants) on the side of a surface element in contrast to the stress concept of force per unit area. Therefore, the constitutive relations characterizing the membrane material behavior will be in the form of resultants vs. deformation and rate of deformation.

For viscoelastic behavior of a plasma membrane, it is necessary to consider the simultaneous elastic free energy storage and internal, viscous dissipation. The simplest model for viscoelasticity is the superposition of elastic resultants and viscous shear resultants (with CGS units of dynes per centimeter).

$$T_{ij} = T_{ij}^e + T_{ij}^v, \tag{1}$$

where the indices (i, j) represent the in-plane membrane coordinates, either 1 or 2 (Fig. 1).[4]

Elastic energy storage is determined by the intrinsic material deformation. The deformation is defined by the Lagrangian strain tensor for shape changes relative to the "metric" of the initial coordinate system (describing the "natural" undeformed state of the material). Choosing the principal axis coordinate system (where the deformation of the material is simply extension and compression along these axes), the Lagrangian strain tensor, ϵ_{ij}, can be expressed in terms of the material extension ratios, λ_1 and λ_2.

$$\epsilon_{11} = (\lambda_1^2 - 1)/2,$$

$$\epsilon_{22} = (\lambda_2^2 - 1)/2,$$

$$\epsilon_{12} = \epsilon_{21} = 0. \tag{2}$$

The extension ratios are the ratio of the final material element length to its original, elemental length:

$$\lambda_1 = dx_1/da_1,$$

$$\lambda_2 = dx_2/da_2,$$

where x_i is the ith coordinate in the deformed membrane and a_i is the ith coordinate in the initial state.

The elastic resultant tensor for a two-dimensional, hyperelastic material with large resistance to area change is given by (Evans, 1975 b),

$$T_{ij}^e = (\gamma + K\alpha)\delta_{ij} + \mu\epsilon_{ij}, \tag{3}$$

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[4]Skalak (1973) has introduced an integral equation formulism for considering stress relaxation functions of a two-dimensional material, based on force resultants relative to the initial, unstretched state. The formulism is a linear viscoelastic constitutive relation. However, the large deformations considered in this paper create a nonlinear relationship between the rate of deformation and the time derivative of the strain tensor; therefore, Eq. 1 will not in general be a linear, viscoelastic constitutive relation.
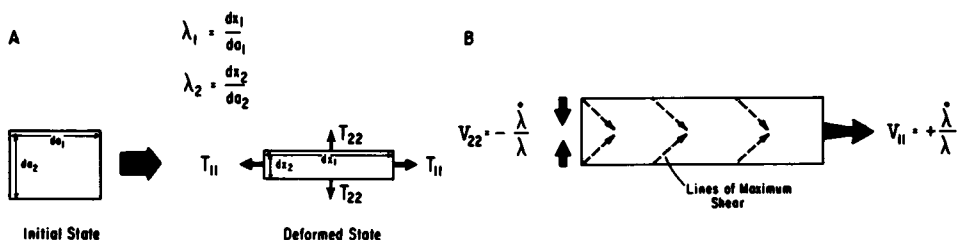
FIGURE 1 Schematic illustration of (A) the deformation of a square membrane material element in the principal axis system and (B) the principal components of the *rate* of deformation of the plane element in extension. The principal tensions, T_{11} and T_{22}, are forces per unit length.

where δ_{ij} is the identity matrix, γ is the interfacial free energy density of hydrophobic interaction, K is an area compressibility modulus, α is the area strain ($\alpha = \lambda_1 \cdot \lambda_2 - 1$), and μ is a modulus of rigidity or shear modulus. Because the area compressibility is very small (i.e. K is large compared with μ), the material can be treated as two-dimensionally incompressible and the first term can be replaced by an isotropic tension analogous to hydrostatic pressure in bulk liquids:

$$T_{ij}^{e} = -P_{m}\delta_{ij} + \mu\epsilon_{ij}. \tag{4}$$

The constants (γ, K, μ) have units of surface energy density (ergs/cm²) or tension (dyn/cm). This relation has been used satisfactorily to analyze large elastic deformations in red cell membranes produced by micropipette suction and fluid shear deformation of attached cells (Evans, 1973 *b*).

The viscous shear resultant in Eq. 1 can be expressed (to first order) as a constant times the rate of deformation tensor (Evans, 1975 *b*),

$$T_{ij}^{v} = 2\eta_{e}V_{ij}, \tag{5}$$

where η_{e} is the parameter characterizing the viscous dissipation during elastic deformations at finite rates and η_{e} is a surface viscosity (dyn-s/cm). In general, the viscous material constant, η_{e}, would depend both on the material deformation and the rate of deformation. In the Appendix, we consider a hypothetical situation where the viscous parameter varies as the square of the principal extension ratio for an element in uniaxial tension. The physical reasoning behind such a consideration is discussed in the Appendix.

The rate of deformation tensor, V_{ij}(second⁻¹), is specified by the time rate of change of the square of the metric in the instantaneous or deformed material coordinate system. The difference between the square of the metric in the deformed and initial coordinate systems defines the Lagrangian strain tensor.

$$ds^{2} - ds_{0}^{2} = dx_{k}dx_{k} - da_{k}da_{k} \equiv 2\epsilon_{ij}da_{i}da_{j}. \tag{6}$$

Thus,

$$\epsilon_{ij} \equiv \frac{1}{2} [(\partial x_k / \partial a_i)(\partial x_k / \partial a_j) - \delta_{ij}],$$

(where the Einstein summation convention for repeated indices is used). Taking the rate of change of the metric Eq. 6 gives the rate of deformation tensor in terms of the time rate of change of the Lagrangian strain tensor:

$$[(\partial v_i / \partial x_j) + (\partial v_j / \partial x_i)] \, dx_i dx_j = 2\dot{\epsilon}_{ij} da_i da_j,$$

$$2V_{ij} \equiv (\partial v_i / \partial x_j) + (\partial v_j / \partial x_i), \tag{7}$$

where (˙) denotes the partial derivative with respect to time and v_i is the ith component of the in-plane velocity field, $\dot{x}_i$. For an arbitrary choice of metric components, Eq. 7 becomes,

$$V_{ij} = \dot{\epsilon}_{kl}(\partial a_k / \partial x_i)(\partial a_l / \partial x_j). \tag{8}$$

In the principal axis system,

$$dx_1 / da_1 = \lambda_1 \quad \text{and} \quad dx_2 / da_2 = \lambda_2.$$

Then, from Eqs. 2 and 8, the components of the rate of deformation tensor are given by,

$$V_{11} = \dot{\lambda}_1 / \lambda_1 = \partial v_1 / \partial x_1,$$

$$V_{22} = \dot{\lambda}_2 / \lambda_2 = \partial v_2 / \partial x_2. \tag{9}$$

(The principal axis system orientation is assumed not to change with time.) The condition of two-dimensional incompressibility (constant element area) is defined by $\lambda_1 \cdot \lambda_2 = 1$. Therefore, the time rate of change of the extension ratio in one direction is simply related to the time rate of change of the other extension ratio:

$$\dot{\lambda}_1 / \lambda_1 + \dot{\lambda}_2 / \lambda_2 = 0. \tag{10}$$

Therefore,

$$V_{11} + V_{22} = 0,$$

which defines the two-dimensional incompressibility condition in Eulerian variables,

$$\partial v_1 / \partial x_1 + \partial v_2 / \partial x_2 = 0.$$

This is the continuity equation for a two-dimensional, incompressible fluid.

By combining Eqs. 1, 2, 4, 5, and 9, the membrane tension in an element undergoing simple extension (in the principal axis system), can be written as,

$$T_{11} = -P_m + (\mu/2)(\lambda_1^2 - 1) + 2\eta_e(\dot{\lambda}_1 / \lambda_1),$$

$$T_{22} = -P_m + (\mu/2)(\lambda_2^2 - 1) + 2\eta_e(\dot{\lambda}_2 / \lambda_2). \tag{11}$$

Because $\lambda_1 \lambda_2 \simeq 1$, the component T_{22} can be given by,

$$T_{22} = -P_m + (\mu/2)(\lambda_1^{-2} - 1) - 2\eta_e(\dot{\lambda}_1 / \lambda_1). \tag{12}$$

If the element experiences uniaxial tension, T_{22} would equal zero and the result for T_{11} is simply

$$T_{11} = (\mu/2)(\lambda_1^2 - \lambda_1^{-2}) + 4\eta_e(\dot{\lambda}_1/\lambda_1). \qquad (13)$$

This final relation is a simple, viscoelastic constitutive function that characterizes the time-dependent response of the membrane undergoing finite deformation elastically in uniaxial extension.

MEMBRANE RELAXATION OF A MICROPIPETTE PRODUCED PROJECTION

The aspiration of a red blood cell discocyte into a small micropipette applied along the axis of symmetry is schematically illustrated in Fig. 2. It has been shown that the negative pressure required to produce an equilibrium projection length, D, is related to the elastic deformation of membrane material in the pipette (Evans, 1973 b). The extension ratio along the cylindrical surface generator (meridian) varies between unity at the tip of the projection to a maximum, $\hat{\lambda}$, at the pipette entrance. The maximum extension ratio is uniquely specified by the ratio of the projection length to the radius of the pipette, R_p, shown in Fig. 3. When the cell is expelled from the pipette, the membrane relaxes back to its original biconcave, discoidal shape (provided that the cell is not held in the pipette for periods of time greater than a few minutes—see Evans and LaCelle, 1975, for discussion). The time required for the membrane projection to decrease in length by 50% is about 0.3 s for human red cells. Similar values for the time required for complete cell relaxation were observed when red cells were rapidly expelled from 3–4 μm diameter pipettes (Hoeber and Hochmuth, 1970).

When the cell projection is expelled, the forces present are the internal membrane material resultants (already described) plus the viscous fluid resistance of the cytoplas-
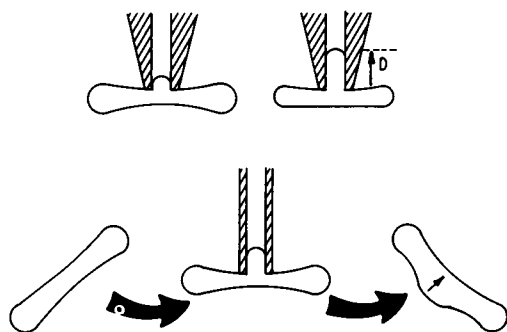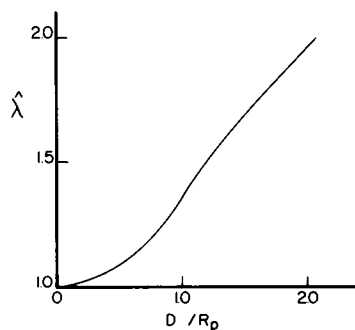

FIGURE 2

FIGURE 3

FIGURE 2 Schematic illustration of the micropipette aspiration of a red cell discocyte and its subsequent expulsion.

FIGURE 3 Plot of the maximum membrane extension ratio, $\hat{\lambda}$ ("degree" of stretch), which occurs at the entrance to the pipette, as a function of the height of the aspirated projection, D, in units of pipette radii, R_p.

mic and extracellular media and the membrane bending moments created by the curvature in the membrane projection. In general, the elastic energy caused by bending is much smaller than the elastic energy produced by shear deformation (Evans, 1974). Therefore, bending effects will be neglected. (However, bending moments are dominant in the region where the projection is continuous with the flat outer membrane surface and will reduce the local curvature at this point). The important consideration is the viscous dissipation in the cytoplasm and extracellular fluids which would create a transmembrane, hydrostatic pressure difference and thus retard the rate of relaxation of the cell projection. Because the red cell hemoglobin solution viscosity is significantly greater than that of the extracellular fluid, only fluid dissipation inside the cell need be evaluated. The maximum value for the rate of energy dissipation in the cytoplasm is estimated by

$$(ergs/s) \ \dot{F}_H \sim \tilde{\eta}_H \, |V|^2 \, (D\pi R_p^2), \tag{14}$$

where $|V|^2$ is the square of the rate of deformation. The quantity inside the parenthesis is the projection volume and $\tilde{\eta}_H$ is the viscosity of the hemoglobin solution (ordinary liquid viscosity in dynes-second per square centimeter). The rate of energy dissipation in the membrane is estimated by

$$(ergs/s) \ \dot{F}_M \sim \eta_e \, |V|^2 \, (D2\pi R_p). \tag{15}$$

Viscous dissipation in the cytoplasmic fluid can be neglected if $\dot{F}_H \ll \dot{F}_M$. The ratio of the dissipations is,

$$(\dot{F}_H/\dot{F}_M) \sim (\tilde{\eta}_H R_p/\eta_e) \tag{16}$$

because the rate of deformation is of the same order in both cytoplasm and membrane. The viscosity of the hemoglobin solution is of the order 10^{-1} dyn-s/cm^2 (Cokelet and Meiselman, 1968) and the pipette radius is of order 10^{-4} cm. Since we will demonstrate that the surface viscosity, η_e, is approximately 10^{-3} dyn-s/cm,

$$(\dot{F}_H/\dot{F}_M) \sim 10^{-2} \quad \text{or} \quad \dot{F}_H \ll \dot{F}_M.$$

Thus, the viscous fluid resistance during the membrane relaxation can be neglected and the transmembrane hydrostatic pressure difference is set equal to zero.

Because no appreciable external forces resist the membrane relaxation, the internal elastic restoring forces must balance the internal viscous (resistance) forces.

$$T_{ij} = T_{ij}^e + T_{ij}^v = 0.$$

Thus, Eq. 13 gives the following time-dependent differential equation for λ_1:

$$(\mu/2)(\lambda_1^2 - \lambda_1^{-2}) + 4\eta_e(\dot{\lambda}_1/\lambda_1) = 0. \tag{18}$$

If we assume that μ and η_e are constants (independent of deformation and rate of deformation),[5] the temporal evolution of the extension ratio λ_1 is given by

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[5] See Appendix for the case of η_e a function of the extension ratio.

$$\left(\frac{\lambda_1^2 - 1}{\lambda_1^2 + 1}\right) = \left(\frac{\bar{\lambda}_1^2 - 1}{\bar{\lambda}_1^2 + 1}\right) e^{-t/t_o} \qquad (19)$$

where $\bar{\lambda}_1$ is the initial value of the extension ratio (degree of stretch along the meridian of the projection), which is recognized to be a function of position along the membrane projection; and t_o is the characteristic time constant for the membrane material

$$t_o \equiv 2\eta_e/\mu. \qquad (20)$$

Eq. 19 is used to calculate, with a digital computer, the temporal relaxation of a membrane projection. The computer is required because the extension ratio is a function of location on the projection. The relaxation equation 19 specifies the change in the material extension, but the shape must be determined by integrating the extension ratio over the surface starting from the top, center of the projection where $\lambda \equiv 1$. Fig. 4 shows an example for an initial D/R_p ratio of 2:1. In the range of lengths defined by D/R_p of 1–3, the time required to relax by 50% (0.5D) was essentially 1.5 t_0 (with only slight variation). The time $t_{D/2}$ obtained from Eq. 20 is,

$$t_{D/2} = 3\eta_e/\mu, \qquad (21)$$

and the surface viscosity characterizing viscoelastic deformation is,

$$\eta_e = \mu(t_{D/2})/3$$

Using 10^{-2} dyn/cm for the shear modulus (Evans and LaCelle, 1975; Hochmuth and Mohandas, 1972) and 0.3 s for $t_{D/2}$ from micropipette experiments, the viscosity coefficient, η_e, is of the order,
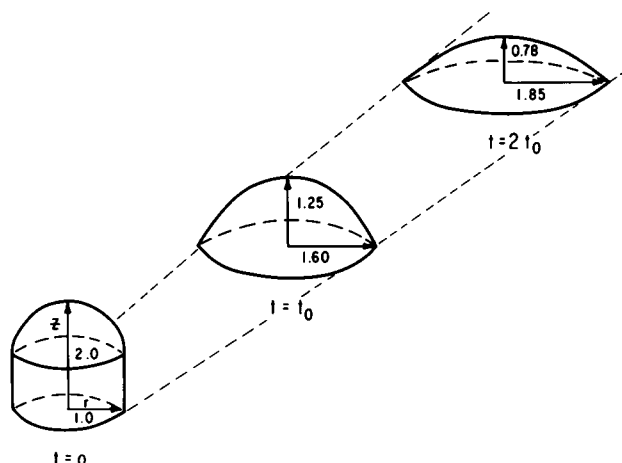

FIGURE 4 Calculated relaxation of the aspirated cylindrical projection (plus hemispherical cap) after expulsion from the pipette for an initial height of D/R_p = 2 and for a constant membrane "viscosity," η_e. The time for each shape is given in units of the characteristic time, $t_0 \equiv 2\eta_e/\mu$.

$$\eta_e \approx 10^{-3} \text{ dyn-s/cm}.$$

In the companion article on membrane viscoplastic flow, it is shown that the membrane viscosity coefficient above the yield shear (in plastic flow) is also of the order 10^{-2} dyn-s/cm, as determined by microtether growth produced by fluid shear deformation of point attached red cells. The surface viscosity determined from "fluidity" measurements of the lipid membrane components (see Edidin, 1974, for a review of these measurements) is of the order 10^{-5} dyn-s/cm.[6] It is apparent that the lipid bilayer contributes little to the fluid dynamic behavior of the whole plasma membrane and that an additional structural matrix must exist to account for the membrane mechanical behavior. This is the same conclusion as that obtained from our measurements of the membrane shear modulus since μ is zero for a lipid bilayer (see Evans and LaCelle, 1975; and Evans, 1975a).

The structural matrix may play an extensive role in the resistance to isotropic tension as well (e.g. in osmotic lysis); cohesive forces at matrix "cross-link" locations could account for the large difference between the isotropic tension required to produce lysis and the ultimate strength of a lipid bilayer (determined by the hydrophobic interaction density γ) (Evans, 1975a). On the other hand, the viscous dissipation in the structural matrix cannot account for the time dependence of the tension producing lysis. From Eq. 5, we see that the membrane viscous tension (resistance) is proportional to the rate of deformation times the surface viscosity. In lysis (failure in isotropic tension), the rate of deformation is less than 10^{-2} s^{-1} because the area increase of a few percent takes place over time periods of 10–100 s (Rand, 1964). The viscous tension would only be 10^{-5} dyn/cm, considerably less than the range of 5–20 dyn/cm reported by Rand (1964). As pointed out in a previous footnote, the small rate of deformation is not indicative of resistance to "bulk" movement in the membrane surface, and such a presumption has resulted in the unusually large estimates of surface viscosities calculated from lysis experiments by Rand and Katchalsky et al. Those estimates are *six* to *eight* orders of magnitude greater than η_e (the viscosities estimated from isotropic tension failure give values in the range 10^3–10^4 dyn-s/cm).

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APPENDIX

The viscoelastic parameter, η_e, that characterizes viscous energy dissipation in the membrane can be a function of both the rate of deformation and deformation (Meares, 1965; Treloar, 1967; Tobolsky and Mark, 1971). For a solution of long chain, flexible molecules or a "loose" network of these molecules, kinetic theory provides approximations for the viscosity dependence on the size and shape of the individual chains (Tobolsky and Mark, 1971; Treloar, 1967). The viscosity is proportional to square of the radius of gyration of the long chain molecule. The radius of gyration of subunits in a loose network would change in proportion to the change in the metric as the material is deformed (provided that the subunit is essentially, randomly arranged in the initial or "natural" state). Therefore, an interesting hypothetical case is to
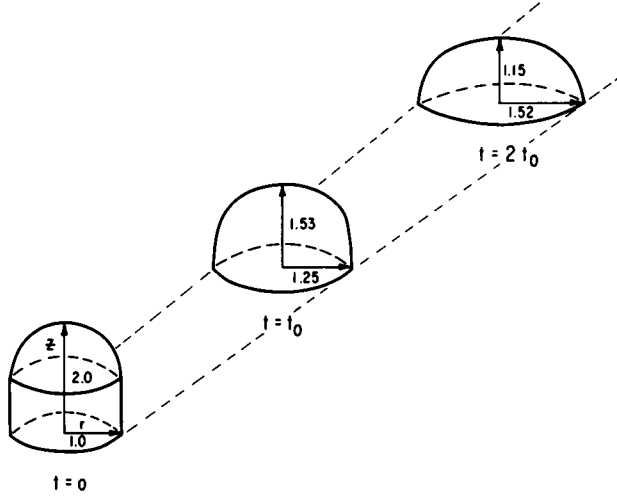
FIGURE 5 Calculated relaxation of the aspirated projection after expulsion from the pipette for an initial height of $D/R_p = 2$ and for the *hypothetical* case of membrane "viscosity" proportional to the square of the extention ratio. The time is given in units of the characteristic time, $t_0 = 2\eta_e^o/\mu$.

consider the viscous parameter to be proportional to the square of the metric change,

$$\eta_e \sim (ds/ds_0)^2 = (\lambda_1^2 + \lambda_1^{-2})/2.$$

The viscosity relation can be approximately treated by,

$$\eta_e = \eta_e^o \lambda_1^2,$$

where λ_1 is the extension ratio in the direction of stretch. Using this relation for viscosity in Eq. 18 gives a different relation for relaxation of the stretch ratio λ_1,

$$(\lambda_1^4 - 1) = (\bar{\lambda}_1^4 - 1)e^{-t/t_0},$$

where the material time constant, t_0, is defined by,

$$t_0 \equiv 2\eta_e^0/\mu.$$

The relaxation of a membrane projection in this case is shown in Fig. 5. The time required to reach one-half the initial projection height, $D/2$, was found to be $\sim 2.5\ t_0$. The viscous parameter η_e^o is, in this case,

$$\eta_e^o = \mu(tD/2)/5.$$

The surface viscosity would vary between η_e^o at the top to about $4\eta_e^o$ at the bottom of the projection for the example shown.