Membrane Elasticity Effects on Permeability Measurements in Vesicles

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Typical experiments to measure permeability in biologically relevant cell membranes utilize vesicles, single bilayer membrane capsules, as idealized cells. The permeation of solute through the vesicle membrane is analyzed with the assumption that the vesicle volume and membrane area are fixed during the experiment. In response to the excess solute concentration internal to the vesicle, however, an osmotic stress is set up that acts to stretch the vesicle membrane, resulting in an initial dilution of the vesicle contents, followed by a continued elastic response of the vesicle membrane as the experiment proceeds and the internal and external concentrations equilibrate. We derive a simple analytic theory of this process that couples the mass-transfer process with the area elasticity of the vesicle membrane. If dilution of the exterior solution is used to initiate the permeation, significant effects on measured permeabilities are predicted for membranes with a low elastic modulus, when large concentration differences are imposed or vesicles are greater than 0.1 micron in diameter. In practice, this leads to an underestimate of the permeability of membranes composed of polyunsaturated lipids or lipids in contact with alcohols or alkanes. It could also lead to the erroneous conclusion that the permeability is concentration-dependent. The effects of elasticity, however, are minor, if dialysis is used to continually remove any exterior solute or if saturated phospholipid membranes with a large elastic modulus or vesicles of less than 0.1 micron diameter are used.

More than a quarter of a century ago, Bangham and coworkers (1967) showed that phospholipids dispersed in water formed closed, multibilayer aggregates called vesicles or liposomes, which are capable of separating an internal compartment from the bulk solution. These phospholipid and synthetic surfactant bilayers have osmotic and elastic properties similar to those of biological cell membranes and have been widely used as cell models (Papahadjopoulos and Miller, 1967; Huang, 1969; Evans and Skalak, 1980; Evans and Needham, 1987). Physico-mechanical properties, such as membrane area elasticity, membrane bending rigidity, critical tension for bursting (lysis), and membrane water and solute permeability, are important parameters in many cellular processes and functions like nutrient transport, cell-waste disposal, hemolysis, cell membrane adhesion and fusion and other immunological processes (Weiss, 1968; Ponder, 1971; Evans and Skalak, 1980; Zimmerberg et al., 1980; Bailey et al., 1990). Such membrane parameters are much more easily studied in vesicles and liposomes (Evans and Needham, 1987). In addition, as bilayers are relatively impermeable to many ions and nonelectrolytes, it became simple to create small domains of different composition within a bilayer shell, and mimic many of the properties that nature has designed into cells and organelles (Papahadjopoulos and Miller, 1967; Huang, 1969; Fendler, 1983; Ostro, 1987). As a result, there has been great effort devoted to using bilayers as drug delivery vehicles (Fendler, 1983; Ostro, 1987) and microreactors for specialized chemistry (Bhandarkar and Bose, 1990). *Vesicles*, which are single-bilayer closed shells that encapsulate an aqueous interior, have become the preferred structure for use in most applications (Fendler, 1983; Ostro, 1987; Kaler et al., 1989). Uni- and multilamellar lipid and surfactant aggregates are also called liposomes, although vesicle usually refers to unilamellar structures and liposome usually refers to multilamellar structures (Szoka and

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Papahadjopoulos, 1980). Within this classification, there are three categories: multilamellar vesicle (MLV), small unilamellar vesicle (SUV), (<100 nm diameter), and large unilamellar vesicle (LUV) (>100 nm). Recent work (Kaler et al., 1989) has shown that it is possible to create spontaneous, equilibrium vesicles from simple surfactant mixtures, which could lead to new applications.

Vesicles are often used as model systems to study solute permeability through bilayers (Lossen, 1972; Selser et al., 1976; Brunner et al., 1980; Carruthers and Melchior, 1983; Castaing et al., 1992), bilayer elasticity (Servuss et al., 1976; Schneider, Jenkins and Webb, 1984; Milon et al., 1986; Sun et al., 1986; Li et al., 1986; Haines et al., 1987; Miyamoto et al., 1988; Duwe and Sackmann, 1990; Rutkowski et al., 1991), and interactions between bilayer membranes (Evans and Needham, 1987; Servuss and Helfrich, 1989; Bailey et al., 1990). In many of these experiments, concentration differences exist between the inside and outside of the vesicle membrane, leading to an osmotic swelling or shrinkage of the vesicles. This coupling of membrane elasticity and permeability is generally ignored in permeability studies; typically, the vesicle is regarded as a solid-like container of fixed area and volume which resists any such osmotically induced deformations. However, it is clear from the many experimental studies listed above that the vesicle membrane is elastic and does change its area in response to an applied osmotic stress. In this work, we present a first-order mass-transport model that shows the coupling between the membrane permeability, P, and the area elasticity of the vesicle membrane, K. A simple analytical result is obtained for the mass transport from vesicles that leads to significant corrections to measured values of the membrane permeability and to possible new ways of measuring vesicle permeability.

Bilayer membranes respond to in-plane shear stresses as highly viscous liquids. They do, however, resist normal stresses such as an isotropic tension or out of plane bending (Evans and Needham, 1987). The molecules within the bilayer membrane are characterized by an optimal surface (or headgroup) area per molecule dictated by the balance of the opposing forces of van der Waals and hydrophobic attraction and electrostatic and steric repulsion. Deviations from the optimal surface area per molecule increase the free energy of the bilayer and lead to a restoring force proportional to the change in membrane area (Israelachvili, 1992). In addition, fluid bilayers can be treated as thin two-dimensional analogues of linear flexible polymers (Nelson et al., 1989). The resulting random shape conformations of bilayers give rise to an entropic contribution to the free energy of the membrane (Servuss and Helfrich, 1989) that is inversely proportional to the bending rigidity of the bilayer (Helfrich, 1978). The elastic response in the case of area dilation of a bilayer, $\alpha=(A-A_o)/A_o$, is approximated by superposing the forces due to restriction of shape fluctuations and those due to an increase in area per molecule and is given as a function of membrane tension τ (Evans and Rawicz, 1990):

$$\alpha=\frac{k_BT}{8\pi k_c}\ln(1+c\tau A)+\frac{\tau}{K} \tag{1}$$

k_c is the membrane curvature elasticity modulus, K is the area elasticity modulus, c is a constant, k_B is Boltzmann's constant and T is the absolute temperature. Evans and Rawicz (1990) studied large unilamellar vesicles (~20 μm diameter) of different lipid and lipid-cholesterol mixtures using micropipette aspiration technique, under isoosmotic and constant volume conditions. The fractional area was found to increase with ln (τ) for very low tensions (<0.1 dyne/cm). At the higher tensions (0.1 to 10^2 dyne/cm) important in this work, the experimental data were well approximated by the linear term in Eq. 1:

$$\alpha=\frac{\tau}{K} \tag{2}$$

This linear relationship between area elasticity and membrane tension has been verified for a variety of natural and synthetic lipid bilayers (Milon et al., 1986; Sun et al., 1986; Li et al., 1986; Evans and Needham, 1987; Haines et al., 1987; Miyamoto et al., 1988; Duwe and Sackmann, 1990; Rutkowski et al., 1991). From our previous experiments (Bailey et al., 1990), we have shown that shape fluctuations and the resultant repulsive interaction are negligible for small unilamellar vesicles (75–200 nm diameter); hence the linear result is especially appropriate for the model considered here.

Water and solute molecules permeate through bilayers when a concentration gradient is induced across the bilayer. Permeability of water across bilayers is extremely high; a value of 10^{-4} cm/s across lecithin bilayers is a reasonable estimate (Carruthers and Melchior, 1986), and there is good agreement in the values measured by different techniques reported in literature. Permeability of solutes like glucose, sucrose, NaCl, KCl, and so on, through phospholipid bilayers is many orders of magnitudes less; for glucose the values of permeability vary from 10^{-10} to 10^{-13} cm/s (Carruthers and Melchior, 1986; Lossen, 1972). The permeability, P, of the membrane to solutes can be related to the diffusivity of the solute through the membrane and the difference in solubility of the solute between the membrane and the solution (Cussler, 1984):

$$P=\frac{DH}{l} \tag{3}$$

in which D is the solute diffusivity, H is a partition coefficient, and l is the membrane thickness. In most experiments, P is considered to be concentration independent, and as a result, independent of any imposed osmotic pressure. The limiting factor that determines the permeability of most water soluble solutes through bilayers is the apolar nature of the membrane interior. Typically, the partition coefficient of a water soluble solute between the aqueous medium inside and outside the vesicle and the hydrocarbon interior of the bilayer membrane is very small. We do not expect that the "chemical" nature of the membrane interior should change due to the imposed osmotic stress, and hence we expect the permeability should be relatively constant. For the simple lipid membranes we consider here, there are no "pores" or "channels" that could enhance the flux of water soluble molecules as exist in many real cell membranes (Weiss, 1968). The model presented here shows that solution concentration can have an effect on measured solute fluxes, even if the permeability is constant.

Typically, solute permeability is measured by creating a concentration difference between the inside and outside of the vesicle membrane, then monitoring the flux of solute from the vesicle. The much higher permeability of water through the

membrane means that water is in a state of quasi-equilibrium at all times during such solute flux. Hence, a time dependent osmotic pressure is developed across the vesicle membrane, which leads to a tension in the membrane, which subsequently leads to an increase in both the vesicle surface area and volume, depending on the magnitude of the tension and the area elastic modulus. The time dependent changes in vesicle volume and surface area then couple to the mass transport from the vesicle.

For an ideal solution, the osmotic pressure across a vesicle membrane is:

$$\Pi = RT(C_i - C_e) \tag{4}$$

where Π is the difference in pressure between the inside and outside of the vesicle, R is the gas constant, T is the temperature, and $C_i - C_e$ is the difference between the concentrations of the solute on the outside and the inside of the vesicle. The lateral tension, σ, developed by such a pressure difference across a thin spherical shell is (Adamson, 1990):

$$\frac{\Pi r}{2} = \sigma \tag{5}$$

r is the outside radius of the vesicle. Combining Eqs. 2-4, the coupling between an imposed concentration difference, the membrane tension, and the change in membrane area is simply:

$$\frac{RT(C_i - C_e)r}{2} = \sigma = K\frac{(A - A_o)}{A_o} \tag{6}$$

This result has been validated by light scattering studies of the dependence of vesicle radius on imposed concentration difference (Rutkowski et al., 1991). Reasonable agreement for values of membrane area elasticity with those obtained using a micropipette aspiration technique is obtained (Evans and Needham, 1987). Values of K measured by these techniques range from about 50 erg/cm^2 (Miyamoto et al., 1988) to nearly 1,000 erg/cm^2 (Evans and Needham, 1987), depending on the lipid type. Highly unsaturated phospholipids (Evans and Rawicz, 1990), lipids mixed with long-chain alcohols or alkanes (Safinya et al., 1989), and sugar-lipids such as digalactosyldiglyceride (DGDG) (Evans and Rawicz, 1990) have the smallest values of K, while saturated phospholipids mixed with cholesterol (Evans and Needham, 1987) have the largest values of K. The chain packing irregularities and chain flexibility induced by the unsaturated and sugar lipids act to diminish the membrane rigidity relative to the saturated lipids that have fewer packing defects and better order. From measured intermembrane distances and membrane thickness as a function of applied osmotic pressure by X-ray diffraction, LeNevue et al., (1977) and Parsegian et al. (1979) calculated lateral compressibility of bilayer membranes. These values are lower by more than an order of magnitude than those measured by micropipette aspiration reported by Evans and Needham (1987). The effects of membrane elasticity on permeation of solutes from vesicles are most dramatic for small values of K, and hence large area and volume dilations.

Transport Model

We assume that a dilute suspension of spherical unilamellar vesicles is initially chemically and physically homogeneous so that the solute concentration interior and exterior to the vesicle membrane is C_i. The solution exterior to the vesicle is then diluted to a solute concentration C_e, which is then held fixed (either by dialysis or by having a sufficiently small ratio of vesicle internal volume to external solution volume). Fast permeation of water through the vesicle membrane induces an osmotic pressure causing the vesicles to expand from a radius r_i to r_{ii} and an internal solute concentration C_{ii}. The initial state is determined by Eq. 5 above, which can be rearranged to reflect the dilution effect of the vesicle volume and area expansion (Miyamoto et al., 1988):

$$C_{ii} = C_i\left(\frac{r_i^3}{r_{ii}^3}\right) \tag{7a}$$

$$r_{ii} - r_i = \frac{r_{ii}r_i^2RT}{2K(r_{ii} + r_i)}(C_{ii} - C_e) \tag{7b}$$

which for small expansions can be approximated by:

$$r_{ii} - r_i = \frac{r_i^2RT}{4K}(C_{ii} - C_e) \tag{7c}$$

After the initial swelling with water, the solute begins to permeate from the vesicle membrane with a temperature and concentration independent permeability, P:

$$\frac{d(CV)}{dt} = -AP(C - C_e) \tag{8}$$

in which V is the time-dependent vesicle interior volume, A is the time-dependent vesicle area, and C is the instantaneous solute concentration within the vesicle. Substituting for the vesicle volume and surface area in terms of the vesicle radius and rearranging:

$$\frac{r}{3}\frac{dC}{dt} + C\frac{dr}{dt} = -P(C - C_e) \tag{9}$$

The second term on the lefthand side of the equation is often assumed to be negligible, which is equivalent to assuming that the vesicle has constant volume or a very high area elastic modulus, K. The solution in this case is an exponential decay of concentration difference with time:

$$\frac{C - C_e}{C_i - C_e} = \exp\left(\frac{-3Pt}{r_i}\right) \tag{10}$$

C_i and r_i maintain their initial, unswollen values in this situation. In the general case, Eq. 6 can be used to relate the change in radius to the change in concentration difference. Let X, Y and τ be the appropriate dimensionless concentration difference, vesicle radius and time:

$$X = \frac{C - C_e}{C_i - C_e} \quad Y = \frac{r - r_i}{r_i} \quad \tau = \frac{-3Pt}{r_i} \tag{11}$$

In terms of these new dimensionless variables Eqs. 9 and 6 are:

$$(1+Y)\frac{dX}{d\tau}+3(\beta+X)\frac{dY}{d\tau}=-X, \text{ in which } \beta=\frac{C_e}{C_i-C_e} \quad (12)$$

$$X=\Delta\left[(1+Y)-\frac{1}{(1+Y)}\right], \text{ in which } \Delta=\frac{2K}{RT(C_i-C_e)r_i} \quad (13)$$

Eliminating X in Eq. 12 using Eq. 13 gives:

$$\left[4(1+Y)^2+3\frac{\beta}{\Delta}(1+Y)-2\right]\frac{dY}{d\tau}=1-(1+Y)^2 \quad (14)$$

Equation 14 represents the transport model for solute permeability P explicitly in terms of the vesicle radius r and the membrane elasticity K. It can be solved for the initial condition given by $r=r_{ii}$ at $t(\tau)=0$ or in terms of $Y_i=(r_{ii}-r_i)/r_i$:

$$4(Y-Y_i)+\frac{3\beta}{2\Delta}\ln\left[\frac{Y(2+Y)}{Y_i(2+Y_i)}\right]+\ln\left[\frac{Y(2+Y_i)}{Y_i(2+Y)}\right]=-\tau \quad (15)$$

and from this expression, Y can be solved as a function of time. Once $Y(\tau)$ is known, then $X(\tau)$ can be evaluated using Eq. 13.

For reasonable experimental parameters, vesicles swell no more than about 3-10% of their initial radius before lysis (Evans and Needham, 1987). Hence, $Y_i \ll 1$ and $|\ln(Y)| \gg Y \gg Y^2$ and Eq. 15 can be approximated by:

$$\frac{Y}{Y_i}=\exp\frac{-\tau}{1+\frac{3\beta}{2\Delta}} \quad \text{or} \quad \frac{r-r_i}{r_{ii}-r_i}=\exp\frac{-3Pt}{r_i\left(1+\frac{3C_eRTr_i}{4K}\right)} \quad (16)$$

To an equal approximation, X is given as:

$$X=\Delta\left[(1+Y)-\frac{1}{1+Y}\right]\approx 2\Delta Y \quad (17)$$

which gives a simple explicit form for X as a function of time:

$$X=\frac{C-C_e}{C_i-C_e}=\left[\frac{4K}{RT(C_i-C_e)r_i^2}(r_{ii}-r_i)\right]\exp\frac{-3Pt}{r_i\left(1+\frac{3C_eRTr_i}{4K}\right)} \quad (18)$$

using Eq. 7c this becomes:

$$\frac{C-C_e}{C_i-C_e}=\left[\frac{C_{ii}-C_e}{C_i-C_e}\right]\exp\frac{-3Pt}{r_i\left(1+\frac{3C_eRTr_i}{4K}\right)} \quad (18a)$$

and the simple exponential form of Eq. 10 is recovered, except the argument of the exponential is reduced by the membrane elasticity, which is always positive or zero, and hence always reduces the permeation of solute from the vesicles. A surprising result of the linearized form is that for very small external concentrations, $C_e \approx 0$, the argument of the exponential is independent of membrane elasticity and reduces to Eq. 10 above. Only the prefactor reflects the initial swelling of the vesicle.

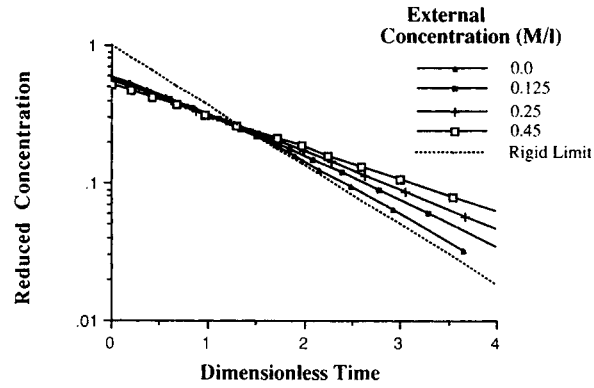

Figure 1. Effect of the external concentration, hence the dilution of the vesicle exterior, for a fixed value of 0.5 M/L for the initial internal concentration, C_i, a value of 50 dyne/cm for membrane elasticity, K, and an initial radius, r_i of 50 nm.

As the external concentration decreases from 0.45 M/L to 0 M/L, the solution approaches the rigid limit, as expected from Eq. 18a.

Results and Discussion

Figures 1-4 show plots of vesicle internal concentration derived from the exact solution, Eqs. 13 and 15, vs. time on semi-log plots. Figure 1 shows the effect of the external concentration, hence the dilution of the vesicle exterior, for a fixed value of 0.5 M/L for the initial internal concentration, a value of 50 dyne/cm for membrane elasticity, and an initial radius of 50 nm. As the external concentration decreases from 0.45 M/L to 0 M/L, the solution approaches the rigid limit, as expected from Eq. 18a. Figure 2 shows the effect of membrane elasticity, K, for fixed values of 0.5 M/L for the initial internal concentration, 0.25 M/L for the external concentration, and 50 nm for the initial radius. As expected, the deviation from

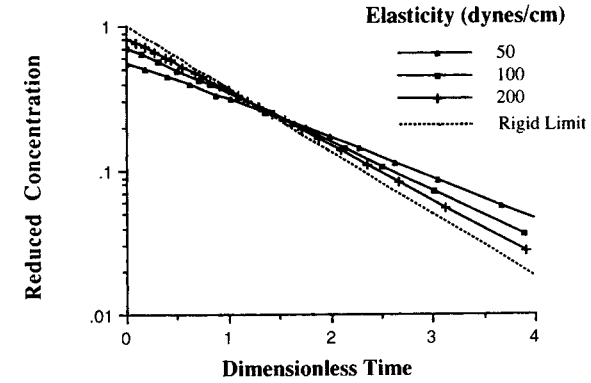

Figure 2. Effect of membrane elasticity, K, for fixed values of 0.5 M/L for the initial internal concentration, 0.25 M/L for the external concentration, and 50 nm for the initial radius.

As expected, the deviation from the rigid limit increases as the membrane elasticity decreases.

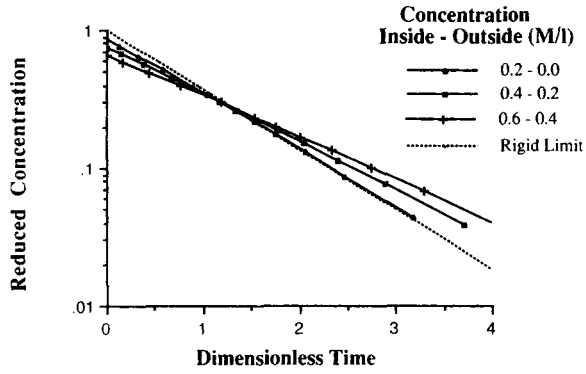

Figure 3. Permeability depends not only on the magnitude of the concentration difference, but on the initial internal and external concentration.

For a concentration difference of 0.2 M/L, there is significant deviation from the rigid limit for an external concentration of 0.4 M/L, but virtually none for a zero external concentration.

the rigid limit increases as the membrane elasticity decreases. Figure 3 shows that the permeability depends not only on the magnitude of the concentration difference, but on the initial internal and external concentration. For a concentration difference of 0.2 M/L, there is significant deviation from the rigid limit for an external concentration of 0.4 M/L, but virtually none for a zero external concentration. Figure 4 shows that for a membrane elasticity of 100 dyne/cm, an initial internal concentration of 0.5 M/L, and an external concentration of 0.25 M/L, the deviation from the rigid vesicle limit is greatest for larger vesicles. Except for extreme values of the parameters, the results are linear, in good agreement with the approximate solutions, Eqs. 16 and 18. This is consistent with experimental results, which usually show an exponential decrease in concentration with time.

In a typical experiment, the concentration of solute (or of a solute label) is monitored as a function of time. The logarithm of the concentration is plotted vs. time, and the slope of the line, S, is interpreted according to Eq. 10 above to be equal to $-3P/r_i$. Hence, $P=-r_iS/3$. This assumes that no swelling of the vesicles occur in response to the imposed concentration

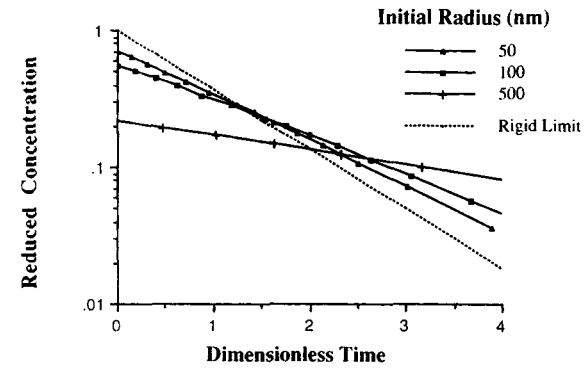

Figure 4. For a membrane elasticity of 100 dyne/cm, an initial internal concentration of 0.5 M/L, and an external concentration of 0.25 M/L, the deviation from the rigid vesicle limit is greatest for larger vesicles.

gradient. For reasonable values of the membrane elasticity, K, and the concentration difference, however, the slope, S, is reasonably approximated by:

$$\frac{-3P}{r_i\left(1+\frac{3C_eRTr_i}{4K}\right)} \tag{19}$$

and the permeability is

$$P=\frac{-r_iS}{3}\left(1+\frac{3C_eRTr_i}{4K}\right).$$

Therefore, the permeabilities are actually greater by the factor

$$\left(1+\frac{3C_eRTr_i}{4K}\right),$$

and the membrane permeability can be seriously underestimated, depending on the relative size and elasticity of the vesicle and the choice of internal and external concentrations. For the brush border membrane studied by Miyamoto et al. (1988), K is 80 erg/cm^2. Hence, for a 100-nm radius vesicle and in a medium of 0.5-M concentration, the permeability is underestimated by a factor of 2.2! However, for saturated dimyristoylphosphatidylcholine, with $K=145$ erg/cm^2 (Evans and Needham, 1987), a vesicle of 50-nm radius, in a medium of concentration 0.1 M, the permeability is underestimated by only a factor of 1.1, which is probably within the error of a typical experiment. Hence, significant effects on measured permeabilities are predicted for membranes with a low elastic modulus, large vesicles, or if large concentration differences are imposed. In practice, this might lead to an underestimate of the permeability of membranes composed of polyunsaturated lipids or lipids in contact with alcohols or alkanes.

However, if dialysis is used to bring the concentration of solute external to the vesicle down to near zero ($C_e=0$), then we recover Eq. 10, and from a measure of the slope, S, it is as if the vesicles did not swell at all. However, as shown in the figures, swelling does occur and is appreciable for this choice of parameters. However, when the membrane swells with water, there are two opposing effects that alter the mass transfer. First, the concentration difference driving force is reduced as the internal solute concentration is reduced by dilution. Second, the area available for mass transfer is increased as the membrane area increases. For reasonably small values of swelling, however, these opposing effects cancel and the result is identical to the rigid vesicle case. Hence, for experiments done using dialysis the values determined from the rigid vesicle model are correct.

Finally, this connection between elasticity and permeability suggests new experimental approaches to measuring membrane permeability. For membranes with relatively low values of K, it should be possible to measure the average swelling of a population of vesicles with quasi-elastic light scattering following an imposed concentration gradient. This has been done for a variety of lipids by several groups to measure K (Milon et al., 1986; Sun et al., 1986; Li et al., 1986; Haines et al., 1987; Miyamoto et al., 1988; Rutkowski et al., 1991). After K is measured by recording the initial swelling response, the

permeability could be measured by following the radius of the vesicles as a function of time. The data could be fit to Eq. 15 to extract the membrane permeability. Hence, a single experiment could measure two important membrane properties simultaneously and fairly easily. The major requirement is a method of creating a population of monodisperse vesicles of appropriate size. Commercial equipment for creating such vesicles already exists (Lipex Biomembranes, Vancouver, B.C.), and we plan such experiments for the near future. For large values of K, however, small swellings or polydisperse vesicles, quasi-elastic light scattering is not sufficiently precise for this type of measurement. Care must also be taken not to impose such a large concentration gradient that lysis results. Our theoretical results also suggest that the largest vesicles should be used, as they lead to a larger proportional increase in radius.

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