Entropic Expulsion in Vesicles

Roy Bar-Ziv,[1] Thomas Frisch,[2] and Elisha Moses[1]
[1]*Department of Physics of Complex Systems, Weizmann Institute of Science, Rehovot 76100, Israel*
[2]*Department of Chemical Physics, Weizmann Institute of Science, Rehovot 76100, Israel*
(Received 24 April 1995)

We report experimental observations and theoretical analysis of entropy driven expulsion in giant lipid vesicles, produced by the application of optical tweezers. The tweezers induce tension in membranes by attracting lipid material into the optical trap. This suppresses fluctuations in the vesicles and converts them into tense pressurized spheres. After shutting off the laser, overpressurized vesicles *spontaneously* expel inner vesicles, releasing their inner pressure.

PACS numbers: 68.10.–m, 47.20.Dr, 82.65.Dp, 87.22.Fy

Rupture phenomena and the transfer of smaller vesicles through the membrane wall are central issues in understanding the role of vesicles as receptacles for the contents of cells. While in a biological membrane we expect binding by chemical receptors, adhesion, and fusion to play a key role in transmembrane transport, in artificial vesicles we can hope for a simplified transport model to emerge. This kind of simplified transport model to emerge. This kind of simplified approach has had remarkable success in explaining the shape of both artificial and biological vesicles over the past two decades [1–3]. Investigation of their rupture instabilities and transmembrane motion has until now remained intriguing but largely uncontrollable.

In this work we exploit our ability to vary the tension of a vesicle by external means to induce shape transitions and the expulsion of internal "organelles" from within the vesicle. The actual existence of tension in vesicles and in membrane structures in general is subtle, since it stems from indirect entropic contributions of bending modes, or wrinkles [4–6]. In our case we use the optical tweezers [7] technique to suppress fluctuations and to reduce the area available for this wrinkling at all scales. This converts flaccid, fluctuating vesicles to taut spheres, and causes the expulsion of internal vesicles that are floating around inside the pressurized vesicle. We proceed to develop a theoretical model of how this happens, which indicates that the vesicle becomes over pressurized by an entropic mechanism and consequently expels its contents.

We used standard experimental techniques [8] to produce fluid bilayer membranes in water from dimyristoyl-phosphatidylcholine (DMPC) and digalactosyl-diglyceride (DGDG) (purchased from Sigma). The sample (50 μm thick) is positioned in a temperature regulation stage with long term stability of 10 mK. All experiments were performed in the fluid state of the membranes, typically at 45 °C. We varied the temperature over the range from 26 to 85 °C and consistently obtained expulsion. Our experimental apparatus is based on an inverted optical microscope (Zeiss, Axiovert 135TV) in a commonly used setup [9,10]. An expanded parallel Gaussian 488–514 nm laser beam (Coherent, Innova 70) is brought into the back aperture of a strongly focusing phase contrast objective lens (Zeiss, Planapochromat Ph3, 63×, n.a. 1.4). The laser power input ranged from 20 to 100 mW.

The typical effect of the tweezers on an initially flacid vesicle is shown in Fig. 1. The shape fluctuations characteristic of a floppy vesicle are shown in Figs. 1(a)–1(c) over a few minutes. The laser tweezers are then applied to the vesicle, Figs. 1(d) and 1(e), and during a minute or so the fluctuations decrease until they are no longer apparent, Fig. 1(f). At this point the vesicle has become quite taut, indicating that a non-negligible tension $\sigma \geq 10^{-3}$ erg/cm^2 [6] is present in the membrane. In comparison, using *mechanical* manipulation of very large vesicles (where we avoid tension effects by having sufficient reservoirs of area) we found that a flaccid vesicle has inherent entropic tension as low as $\sigma = 10^{-6}$ erg/cm^2, in agreement with previous measurements [6]. In practice, the pressurization by the laser tweezers is simple in only nearly spherical vesicles. For discocytes [11], if the pressurization is not done very slowly then an instability in the form of "pearling" [12–14] will occur.

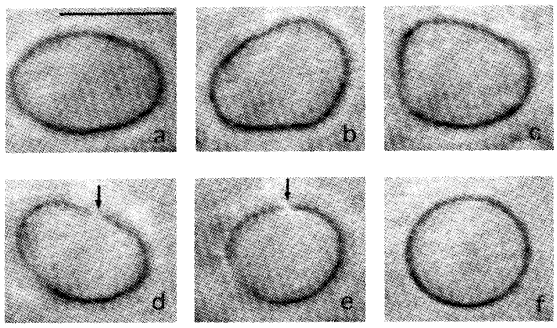

FIG. 1. *Optically induced pressure in a vesicle.* The vesicle was seen to fluctuate strongly at first (a)–(c), then as the optical tweezers were applied (d),(e) the fluctuations gradually decreased until the vesicle became tense (f). It took about 100 sec for the pressurization to be completed. The pressurized vesicle was observed to be unchanged over several hours. The bar represents 10 μm.

0031-9007/95/75(19)/3481(4)$06.00

Actual motion through the membrane is observed when organelles reside within the vesicle. A single pressurized spherical vesicle seems stable for as long as we observed (over many hours). However, vesicles containing internal vesicles which are not too big are unstable and expel their contents. Pressurizing a complicated series of vesicles, contained in each other, we observe a succession of transitions in which the inner vesicles are expelled and the whole structure simplifies. An example of this instability is shown in Fig. 2, where the tweezers pressurized both the inner and outer membranes, and were then turned off. The internal vesicle then drifts until it touches the outer membrane, at which point it probably waits for a large enough fluctuation, then pierces through it and exits. The expulsion occurs outside the plane of focus, and so disappears between Figs. 2(e) and 2(f).

Expulsion has been reported to occur in other instances, and is studied in the context of drug delivery, damage processes, and transport mechanisms in biological cells. Giant vesicles pressurized by the addition of octyl glucoside (OG) exhibit a similar expulsion [15]. The surfactant OG is known to detach mixed lipid-surfactant micelles from the vesicle [16], thus reducing area. Osmotic pressure can also cause expulsion of inner vesicles, while partial polymerization of the membrane causes the shrinkage of vesicle area and subsequently an expulsion of water and any inner vesicles [17].

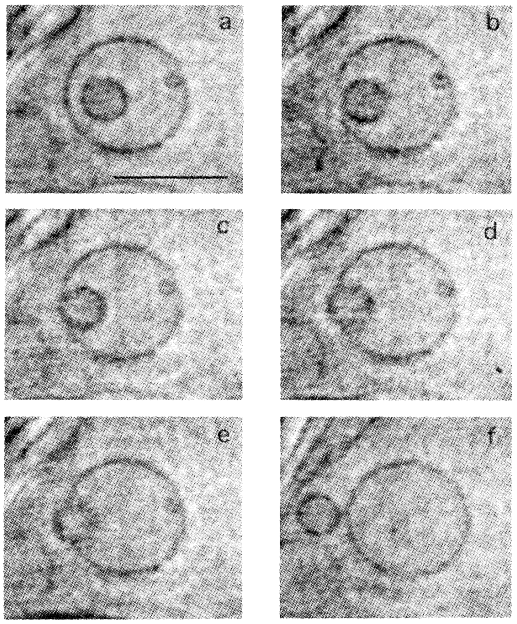

FIG. 2. *Spontaneous expulsion of an inner vesicle.* To reach the configuration in (a), the optical tweezers were used to pressurize a large vesicle and a medium sized vesicle inside it (this initial process is not shown). A very small vesicle inside the right hand region of the large vesicle did not participate in the process. The vesicles can be pressurized independently of each other by focusing on different planes, taking care to tweeze the outer vesicle far from the possible point of exit. *The laser is then turned off.* The inner vesicle diffused for about 30 sec until it reached the edge of the outer vesicle. At this point it was static for a few seconds, until it suddenly, with no external interference, exited through the outer membrane wall in under a tenth of a second. Time in seconds: (a) 0, (b) 0.02, (c) 0.04, (d) 0.1 (e) 0.12, (f) 48. The bar represents 10 μm.

Consider now the effect of the strongly focused electric field of the optical tweezers on the membrane. We quantify the surface tension σ_L that the laser can induce by the electrical energy density per unit area of membrane in the trap. At the laser intensities we use ($\approx$50 mW) this is equivalent to 1×10^{-3} erg/cm^2 [13,14]. For phospholipid bilayers this tension is too weak to actually stretch or compress the area per molecule in the bilayer [4–6]. However, it can fold up the membrane within the trap, thereby gaining electrical energy at the expense of increasing the curvature energy.

From the tension we can build a characteristic length for this buckling process, $l_0 = \sqrt{\kappa/\sigma_L} = \sqrt{\kappa c w_0^2/\Delta\epsilon I d}$ with $\kappa \approx 5 \times 10^{-13}$ erg the bending rigidity, $w_0 \approx 0.3$ μm the trap size, I the laser intensity, $d = 40$ Å the membrane thickness, c the speed of light, and $\Delta\epsilon = 0.23$ the dielectric difference between lipids and water in the visible range. Conversely, by substituting for l_0 the size of the optical trap w_0, we define a critical laser intensity for the buckling to occur that is independent of w_0. We calculated this to be on the order of 20 mW, in agreement with the experimentally observed intensity at which tension effects begin to appear. Since w_0 is proportional to the laser wavelength λ, we find $\sigma_L \sim I/\lambda^2$ indicating that the efficiency of tension production for long wavelength lasers such as the Nd-YAG 1.064 μm laser.

Prolonged application of the tweezers causes irreversible area reduction. This probably results from the detachment of lipids into the solvent, in the form of optically unresolvable tiny vesicles or actual monomers. Since the membrane configuration within the trap is not observed directly, this description is conjectural at this point. Heating effects are ruled out because we measured the absorption of light at the laser's wavelength to be very low (a gross upper limit on the heating is on the order of 1 °C [9,18]).

The folding of the membrane inside the trap takes area from thermal wrinkles outside the trap and reduces them to some extent. This reduction of thermal fluctuations of the vesicle is what actually induces the tension, which for values $\leq$0.1 erg/cm^2 is entropic in its origin [4–6]. The maximal tension which can be induced in the vesicle is limited by the maximal trapping force of the tweezers, which lies in the middle range of this "entropic tension" regime.

Tension in an impermeable vesicle leads to a pressure, and this provides the driving force for the spontaneous

expulsion of inner vesicles (Fig. 2). Pressure will first be released through shape changes, eventually leading to a quasispherical shape, where permeability to water becomes relevant (though the time scales are longer than those for expulsion). In general, elastic forces tend to increase volume and inflate the vesicle to a perfect sphere, while entropic fluctuations tend to reduce the volume by wrinkling the membrane at all scales. Therefore, the lowest free energy state is attained at zero pressure and will have a volume which is slightly less than that of a perfect sphere. Some osmotic pressure may be produced by the capture of lipid molecules within the vesicle. However, even for the extreme case, in which all the discarded area is retained within the vesicle as single molecules, we estimate an upper limit of 1 mM change in concentration across the membrane. We have subsequently checked that such an osmolarity, induced by the addition of glucose, does not produce an appreciable swelling [19].

To quantify the excess energy associated with the high pressure we formulate an equation of state for the vesicle. We perform a calculation similar to the one done by Milner and Safran [5], obtaining the pressure difference $P = P_{\text{in}} - P_{\text{out}}$ as a function of the internal volume V of the vesicle. Since the laser is off during the expulsion, the surface area is fixed and set to $A_0 = 4\pi r_0^2$. The conserved volume must obey $V < V_0 \equiv 4\pi r_0^3/3$, while $V_0 - V$ determines the amount of area available for fluctuations of the membrane. We neglect the compressibility of the membrane, as the laser tension is too low to change the area per molecule [6].

Similarly to Milner and Safran [5] we obtain for the mean thermally averaged volume

$$V(P) = \frac{4\pi}{3} r_0^3 - \frac{k_B T}{\kappa} \frac{r_0^3}{4} \sum_{l>1}^{l=l_{\max}} \frac{2l + 1}{l^2 + l + Pr_0^3/2\kappa}. \tag{1}$$

The upper cutoff $l_{\max} = r_0/d$ rules out fluctuations at scales smaller than the membrane thickness $d = 40$ Å. In the continuum limit, when $d \ll r_0$, Eq. (1) reads

$$V(P) = V_0 \Bigg[1 - \frac{3}{16\pi} \times \frac{k_B T}{\kappa} \ln\left(\frac{(r_0/d)^2 + r_0/d + Pr_0^3/2k}{6 + Pr_0^3/2\kappa} \right) \Bigg]. \tag{2}$$

The divergence of Eq. (2) for $P < -12\kappa/r_0^3$ is associated with an instability of the vesicle as it becomes ellipsoidal [20]. Equation (2) can be easily inverted to give P as a function of V. As seen in Fig. 3(b), for $P > -12\kappa/r_0^3$ the pressure increases monotonically with the volume. This increase is due to the large amount of work needed to flatten out thermal fluctuations. In the limit $V \to V_0$ the pressure divergence indicates the flattening out of *all* thermal fluctuations. This effect is purely entropic, since at zero temperature a vesicle can always be inflated to the perfect sphere. Of course, this divergence is physically unreachable, since the area per molecules changes when the pressure is too high.

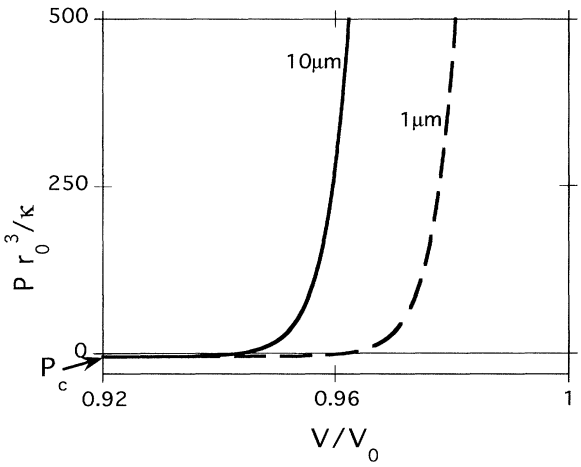

FIG. 3. *Laser induced tension and pressure in vesicles.* The equation of state of pressurized vesicles. Pressure-volume isotherms are given for two relevant vesicle radii.

The volume of water that needs to be expelled from the vesicle to reach zero pressure energy can now be estimated from Eq. (2) as $V(P_{\text{init}}) - V(0)$, with $P_{\text{init}} \approx 2\sigma_l/r_0$. This gives a volume difference of a few percent, in reasonable quantitative agreement with the experiment. Using Eq. (2) we can estimate the energy gain for this expulsion as $\int P(V)\, dV \approx 10^{-11}$ erg. However, the formation of a pore through which the inner vesicle exits is associated with a higher energy cost $2\pi r_1 \gamma \approx 10^{-10}$ erg, where $\gamma \approx 10^{-7}$ erg/cm is the line tension of the pore [21,22]. This leads us to propose that the presence of the inner vesicle reduces the line tension during the exiting process by its interaction with the outer membrane. The microscopic mechanism by which the line tension is reduced in this way is as yet unclear to us and is currently under investigation.

In cases where the inner vesicle (and necessary pore size) is too big, enhancement and acceleration of the expulsion mechanism can only be obtained by the continuous application of the optical tweezers, clamping the inner and outer vesicles together tangentially at a point. Figure 4 shows the first part of a sequence of expulsions which began with three flaccid concentric vesicles, the outer two of which are closely bound and initially indistinguishable, and ended with three separate spherical taut ones. We first induced the formation of a hole in the outer vesicles through which the inner vesicle exited 4(a)–4(d). The hole was produced by local fusion of the two outer vesicles to form a "neck" whose curved lips are observed at the edge of the hole in 4(b)–4(c). The inner vesicle's exit was slow until it reached midpoint, then it was expelled out abruptly. The external vesicles showed visible

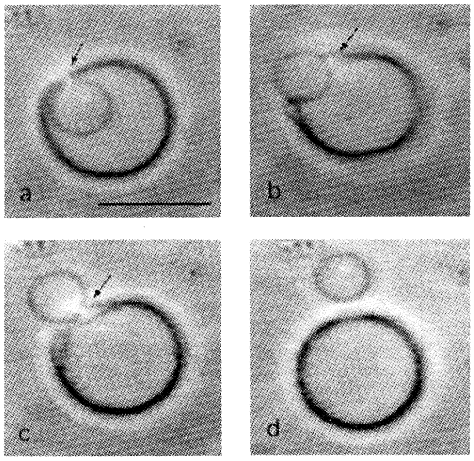

FIG. 4. *Laser controlled expulsion of vesicles.* Time in seconds: (not shown) 0-laser focused to tangency, (a) 0.7, (b) 16.3, (c) 18.3, (not shown) 44-laser turned off, (d) 78. The laser trap is marked by the arrows.

fluctuations (i.e., loss of pressure) even before the inner vesicle reached midpoint, and if the laser were turned off the expulsion would presumably stop.

After the small sphere was expelled we continued to peel off the two concentric vesicles left in the bottom half of 4(d). We could then measure the changes of volume and surface area between the initial 4(d) and final spherical states and found a reduction of $(8.5 \pm 6)\%$ in total volume and a reduction of $(41 \pm 4)\%$ in total surface area. This manifestly shows lipid material is not conserved during tweezing. The detachment of lipid from the membrane into the solution occurs on length scales below our ≈ 0.2 μm resolution.

In conclusion, transmembrane transfer of large organelles can be initiated by entropic pressurization of a vesicle. The time scale for spontaneous expulsion is fast: under $\frac{1}{10}$ of a second. Its existence in biological systems should be detectable by fast measurement techniques. Our ability to quantify the energy release available for pore formation leads us to search for mechanisms by which an inner organelle catalyzes pore nucleation.

We thank A. Ben-Shaul, R. Granek, M. Kraus, V. Lebedev, R. Lipowsky, Z. Olami, and E. Sackmann for fruitful discussions, D. Glaser for a critical reading of the manuscript, and especially P. Nelson and S. Safran for initiating some of the ideas put forth in this paper. Work supported in part by the Minerva Center for Nonlinear Science and the Klutznick Fund for Research.

[1] W. Helfrich, Z. Naturforsch **28C**, 693 (1973).
[2] R. Lipowsky, Nature (London) **349**, 475 (1991).
[3] For a recent review see, for example, X. Michalet, F. Julicher, B. Fourcade, U. Seifert, and D. Bensimon, La Recherche No. 269 **25** (1994).
[4] W. Helfrich and R.-M. Servuss, Nuovo Cimento **3**, 137 (1984).
[5] S. A. Safran, J. Chem. Phys. **78**, 2073 (1983); S. T. Milner and S. A. Safran, Phys. Rev. A **36**, 4371 (1987).
[6] E. Evans and W. Rawicz, Phys. Rev. Lett. **64**, 2094 (1990).
[7] A. Ashkin, Phys. Rev. Lett. **24**, 156 (1970); Science **210**, 1081 (1980); A. Ashkin, J. M. Dziedzic, and T. Yamane, Nature (London) **330**, 769 (1987).
[8] D. Needham and E. Evans, Biochemistry **27**, 8261 (1988); H. P. Duwe, J. Kas, and E. Sackmann, J. Phys. (France) **51**, 945 (1990); M. Mutz and W. Helfrich, J. Phys. (France) **51**, 991 (1990).
[9] S. M. Block, in *Noninvasive Techniques in Cell Biology* (Wiley-Liss, New York, 1990), pp. 375–402; K. Svoboda and S. M. Block, Annu. Rev. Biophys. Biomol. Struct. **23**, 247 (1994).
[10] A. Simon, Ph.D thesis, Univ. of Chicago, June, 1992.
[11] H. J. Deuling and W. Helfrich, Blood Cells **3**, 713 (1977).
[12] R. Bar-Ziv and E. Moses, Phys. Rev. Lett. **73**, 1392 (1994).
[13] P. Nelson, T. Powers, and U. Seifert, Phys. Rev. Lett. **74**, 3384 (1995).
[14] R. Granek and Z. Olami, J. Phys. II (France) **5**, 1348 (1995).
[15] F. M. Menger and K. Gabrielson, J. Am. Chem. Soc., **116**, 1567 (1994); reviewed by J. Emsley, New Scientist **1920**, 14 (1994).
[16] M. Ueno and Y. Akechi, Chem. Lett., 1801-1804 (1991).
[17] M. Dovolaitzky, M. A. Guedeau-Boudeville, and L. Léger, Langmuir **8**, 2595 (1992).
[18] R. Bar-Ziv, R. Menes, E. Moses, and S. A. Safran (to be published).
[19] We thank one of the referees for raising these issues.
[20] Ou-Yang Zhong-can and W. Helfrich, Phys. Rev. Lett. **59**, 2486 (1987); **60**, 1209 (1987).
[21] C. Taupin *et al.*, Biochemistry **14**, 4771 (1975).
[22] W. Harbich and W. Helfrich, Z. Naturforsch **34a**, 1063 (1979).

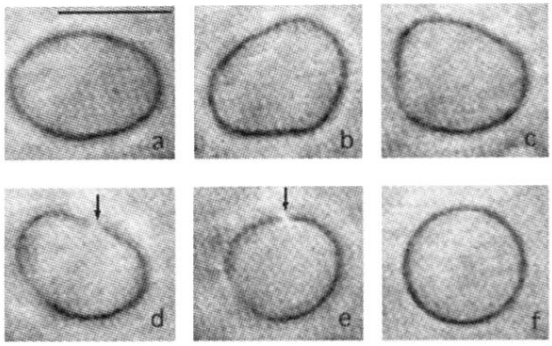

FIG. 1. *Optically induced pressure in a vesicle.* The vesicle was seen to fluctuate strongly at first (a)–(c), then as the optical tweezers were applied (d),(e) the fluctuations gradually decreased until the vesicle became tense (f). It took about 100 sec for the pressurization to be completed. The pressurized vesicle was observed to be unchanged over several hours. The bar represents 10 μm.

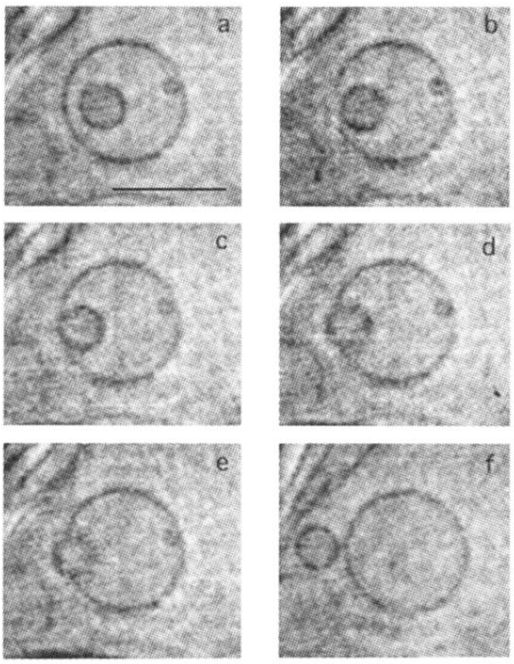

FIG. 2. *Spontaneous expulsion of an inner vesicle.* To reach the configuration in (a), the optical tweezers were used to pressurize a large vesicle and a medium sized vesicle inside it (this initial process is not shown). A very small vesicle inside the right hand region of the large vesicle did not participate in the process. The vesicles can be pressurized independently of each other by focusing on different planes, taking care to tweeze the outer vesicle far from the possible point of exit. *The laser is then turned off.* The inner vesicle diffused for about 30 sec until it reached the edge of the outer vesicle. At this point it was static for a few seconds, until it suddenly, with no external interference, exited through the outer membrane wall in under a tenth of a second. Time in seconds: (a) 0, (b) 0.02, (c) 0.04, (d) 0.1 (e) 0.12, (f) 48. The bar represents 10 μm.

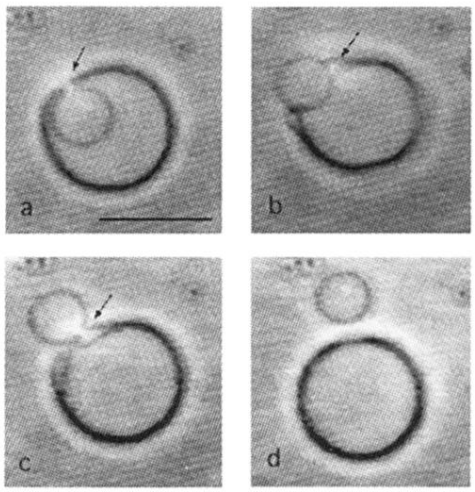

FIG. 4. *Laser controlled expulsion of vesicles.* Time in seconds: (not shown) 0-laser focused to tangency, (a) 0.7, (b) 16.3, (c) 18.3, (not shown) 44-laser turned off, (d) 78. The laser trap is marked by the arrows.